Zero-Valent Iron, Fe (0)-Assisted Autotrophic Denitrification

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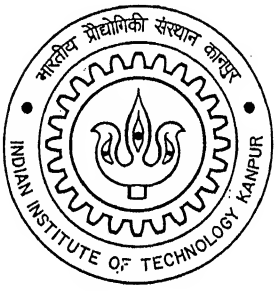

to the

DEPARTMENT OF CIVIL ENGINEERING

Indian Institute of Technology, Kanpur

August, 2002

Dedicated to....

My
Parents, Brothers & other dear Family members

CERTIFICATE

It is certified that the work contained in the thesis **entitled "Zero-Valent Iron, Fe (0) Assisted Autotrophic Denitrification"**, by Mr.Susham Biswas, Roll No. Y011718 has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

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ABSTRACT

Metallic iron-assisted autotrophic denitrification, using hydrogen generated by the anaerobic corrosion of zero-oxidation state iron as the energy source, inorganic carbon as food source, and nitrate as the terminal electron acceptor is a proven phenomenon. This process holds great potential for use in porous 'reactive barriers' containing metallic iron, seeded with autotrophic denitrifying organisms for 'in-situ' remediation of nitrate-contaminated groundwater resources. The main objective of the research described in this dissertation was to determine the optimum composition of the reactive porous medium as described above. This determination is important to ensure that the end-product distribution resulting from nitrate reduction in the reactive media is favorable. In other words, ammonia formation by abiotic nitrate reduction in the presence of metallic iron under anoxic conditions, which is undesirable, has to be minimized, while maintaining a reasonable rate of biological denitrification sustained by hydrogen produced through metallic iron corrosion. Experiments of various types were carried out in a logical progression to achieve this goal. Preliminary experiments included the characterization of autotrophic denitrification supported by externally supplied hydrogen, and by hydrogen generated through anaerobic corrosion of metallic iron. Next, the effect of iron type on abiotic nitrate reduction was studied, and 'steel-wool', with its relatively low surface area to weight ratio, was identified as the iron type exhibiting least propensity to abiotically reduce nitrate. Further, long-term experiments were conducted in batch reactors to determine the effect of 'steel-wool' surface area provided on the extent of denitrification and end product distribution. Finally, long-term denitrification experiments were conducted in up-flow column reactors containing reactive media of various compositions to demonstrate the optimal conditions for biological denitrification in such systems. Based on the results of these experiments, it appears that to achieve acceptable end-product distribution, the 'steel-wool' concentration in the reactive media has to be lowered even below the lowest value, i.e., 0.5 g 'steel-wool' added to 125 cm^3 of acid washed sand, used during this research. This will ensure further reduction in ammonia concentration in the effluent. To counter any detrimental effect of lowered metallic iron concentration on biological nitrate removal, increase of the retention time of water in the reactive media to values higher than 13 days (the maximum value investigated in this study) may be necessary.

CHAPTER I
INTRODUCTION

High concentration of nitrate in potable water can pose a serious health risk. Nitrate has the potential to cause methemoglobinamia both in infants and also in adults deficient in the enzyme glucose-phosphate dehydrogenase (Challis, 1973). Additionally, under rare circumstances, reduction to nitrate in the stomach results in the formation of N-nitroso-amines, which are a group of carcinogenic compounds that have been postulated as a cause of stomach cancer (Mirvish, 1985).

The drinking water standard set by the US. Environmental Protection Agency (Gayle et al. 1990) for nitrate is 10 mg/L (as N). The European Economic Community (EEC) standard is 50 mg/L (as nitrate), or 11.3 mg/L (as N). Indian recommended guideline (Manual of Water Supply and Treatment, 3rd Ed. 1999. pp: 14-15) for concentration of nitrate in drinking water is 45 mg/L (as nitrate).

Various methods have been used all over the world for the removal of nitrate from water. Traditional methods involve passing nitrate-contaminated water through a unit process in a water/wastewater treatment process train for removal of nitrate. The unit processes utilized for nitrate removal can be broadly classified as physico-chemical or biological. Conventional physico-chemical methods for nitrate removal include selective ion exchange, reverse osmosis, and electrodialysis (Hoek et al. 1988). Biological processes involve denitrification reactions utilizing either heterotrophic or autotrophic denitrifying microorganisms for the removal of nitrate.

In recent times, attention has been directed towards the possibility of in-situ remediation of nitrate-contaminated groundwater resources through biological denitrification reactions. Measures proposed for promoting such reactions, or enhancing the biological denitrification rates in subsurface environment include addition of suitable food source and/or electron donor to the nitrate contaminated groundwater. Another approach may be to pass the contaminated groundwater through a 'reactive barrier' constructed in the subsurface across the groundwater flow direction. This barrier is made of porous

material, and is capable of reducing nitrate concentration in groundwater flowing through it.

Recent research reports on 'in-situ' biological denitrification suggest that 'reactive barriers' as described above, and seeded with hydrogenotrophic denitrifying microorganisms may potentially be quite efficient in remediating nitrate contaminated groundwater resources. Hydrogen required as electron donor by these microorganisms may be supplied through anaerobic corrosion of metallic iron embedded in the 'reactive barrier'.

However, before such barriers become practically feasible, certain unresolved questions regarding such barriers need to be addressed. They include, among other things, types of metallic iron that may be suitable and quantity of metallic iron to be used for such applications. Additionally, concerns regarding rates of biological denitrification, microbial inhibition due to pH increase in the barrier, formation of ammonia due to abiotic reduction of nitrate in the presence of metallic iron, and increase in dissolved iron concentration in groundwater need to be investigated. Finally, changes in the long-term performance of such barriers due to changes in barrier porosity due to microbial growth and iron precipitation also need to be investigated.

The main objective of the research described in this dissertation was to determine the optimum composition of the reactive porous medium containing metallic iron and denitrifying microorganisms for removal of nitrate in a flow through system. This determination is important because ammonia formation by abiotic nitrate reduction in the presence of metallic iron, which is undesirable, must be minimized, while maintaining a reasonable rate of biological denitrification sustained by hydrogen production through metallic iron corrosion. As described in the dissertation, experiments of various types were carried out in a logical progression to achieve this goal.

CHAPTER II
LITERATURE REVIEW

2.1 Sources of Nitrate Pollution

One of the main anthropogenic sources for nitrogen pollution in aquatic environment is the nitrogen rich agricultural runoffs from fertilized agricultural fields (Holzmacher et al. 1970, Miller et al. 1974). The applied nitrate loading during fertilization may be as high as 75-300 lbs (as N)/acre (Spalding et al. 1978, Till et al. 1998). In addition, the presence of large amounts of organically bound nitrogen (1500-6000 kg/ha) in the top 150 mm of most agricultural soil is reported (Howard, 1985). The relationship between agricultural activities and nitrate pollution results due to the aeration of the soil during ploughing, resulting in the conversion of a part of organic-nitrogen to nitrate (Young and Gray, 1978). This nitrate, along with a part of nitrate added during artificial fertilization is carried off the field by surface runoff or infiltration, thus polluting the aqueous environment. Fresh water resources viz. streams, rivers and lakes are polluted by surface agricultural run-off, while infiltration of nitrate-rich water to the subsurface results in the pollution of groundwater resources.

Discharge of domestic and industrial sewage into soil or natural aquatic environment is another major anthropogenic source of nitrate pollution (Watson et al. 1967, Franke and McClymonds, 1972). Organic-nitrogen present in such discharges is converted to ammonia-nitrogen by microbial action and may be further converted to nitrate by biological nitrification reactions. As in case of agricultural runoff, surface discharges of wastewater will tend to pollute natural water bodies, while infiltration into soil will cause groundwater pollution. According to the published reports (USGS-OFR, 1990), annual ground water nitrate loading from domestic sewage in Unites States of America (USA) is 4.24 kg/person/year.

2.2 Health Effects

High level of nitrate in potable water can pose a serious health risk. Nitrate has the potential to cause methemoglobinamia, both in infants and also in adults deficient in the

enzyme glucose-phosphate dehydrogenase (Challis et al., 1980). In such cases, the ingested excess nitrate is converted by bacterial reduction to nitrite in the intestinal tract. This nitrate then enters the blood stream and combines with hemoglobin to form methemoglobin, which reduces the capacity of blood to transport oxygen. This disease occurs primarily in infants because the lower acidity of their gastric juices which provide a better environment for nitrate-reducing bacteria (Comley, 1945). Additionally, under rare circumstances, reduction to nitrate in the stomach results in the formation of N-nitrosomines, which are a group of carcinogenic compounds that have been postulated as a cause of stomach cancer (Mirvish, 1992).

2.3 Potable Water Standards for Nitrate

The drinking water standard set by the United States Environmental Protection Agency (USEPA) (Gayle et al. 1989) for nitrate is 10 mg/L (as N). The European Economic Community (EEC) standard is 50 mg/L (as nitrate), or 11.3mg/L (as N). Indian recommended guideline (Manual of Water Supply and Treatment, 3rd Ed. 1999. pp: 14-15) for concentration of nitrate in drinking water is 45 mg/L (as nitrate).

2.4 Environmental Chemistry of Nitrogen

Organic nitrogen contained in dead or decaying plants is converted to ammonia by bacterial action, a process that is known as ammonification. Ammonification reaction may take place both in the presence and absence of oxygen. Ammonia thus produced is converted to nitrite and ultimately to nitrate by nitrification reactions, which take place under aerobic conditions.

Nitrate removal in environment may take place by either assimilatory or dissimilatory pathways. In case of assimilatory reactions, nitrate is consumed by living being as nutrients required for cell synthesis. Some of this nitrate becomes part of the cell and is only released as ammonia on death and decay of the organism as described earlier. Rest of the consumed nitrate is excreted by the organism as ammonia. Thus in case of assimilatory reactions, nitrate is removed by its incorporation into cytoplasmic material and subsequent release of a part of it as ammonia. The dissimilatory reactions for nitrate

removal are also known as denitrification reactions. In such cases, nitrate serves as the electron acceptor in energy metabolism and is converted in to various gaseous end products but principally to molecular nitrogen. Although, denitrification is the primary means by which nitrate removal is achieved, accounting for 70-75% of total removal (Benefield and Randall, 1980), assimilatory nitrate removal is also of primary important in certain cases, especially in marine environments (Koike and Hattori, 1978).

Denitrification reactions, as described above, take place primarily in anaerobic or anoxic environment. In fact, observations have shown that the biological denitrification rate is a decreasing function of oxygen concentration (Nakajima et al. 1984). Both heterotrophic and autotrophic microorganisms have been reported to be responsible for denitrification reactions. Heterotrophic denitrifiers are microorganisms that use organic carbon as the food and energy source and nitrate as electron acceptor for their respiration, thus converting nitrate into nitrogen gas. Equations for denitrification using methanol (Mahony et al., 2000), ethanol (Richard et al., 1980) and acetic acid (Frick et al., 1985) are given as equations 2.1, 2.2 and 2.3 below.

Methanol: $$5CH_3OH+6NO_3^- \rightarrow 3N_2+5CO_2+7H_2O+6OH^- \quad (2.1)$$

Ethanol: $$5C_2H_5OH+12NO_3^- \rightarrow 6N_2+10CO_2+9H_2O+12OH^- \quad (2.2)$$

Acetic Acid: $$5CH_3COOH+8NO_3^- \rightarrow 4N_2+10CO_2+6H_2O+8OH^- \quad (2.3)$$

Autotrophic organisms use inorganic carbon as food source, and electron donors like hydrogen or reduced sulfur-compounds as energy source to reduce nitrate to nitrogen gas. Equations for autotrophic denitrification using hydrogen (Kurt et al., 1987), thiosulfate (Claus and Kutzner, 1985) and sulfide (Barrenstein et al. 1986) as electron donors are given as equations 2.4, 2.5 and 2.6 below.

Hydrogen: $$2NO_3^-+5H_2 \rightarrow N_2+4H_2O+2OH^- \quad (2.4)$$

Thiosulfate: $$5S_2O_3^{2-}+8NO_3^-+H_2O \rightarrow 4N_2+10SO_4^{2-}+2H^+ \quad (2.5)$$

Sulfide: $$5S^{2-}+8NO_3^-+8H^+ \rightarrow 4N_2+5SO_4^{2-}+4H_2O \quad (2.6)$$

2.5 Removal of Nitrate from Water

Various methods have been applied all over the world for the removal of nitrate from water. All these methods involve passing a nitrate-contaminated stream of water through a unit process or process train for removal of nitrate. The processes utilized for nitrate removal can be broadly classified as physico-chemical and biological processes. Biological processes involve denitrification reactions utilizing either heterotrophic or autotrophic denitrifying microorganisms for the removal of nitrate.

In recent times, attention has been directed towards the possibility of in-situ remediation of nitrate-contaminated groundwater resources through biological denitrification reactions. Measures proposed for promoting such reactions, or enhancing the denitrification rates in subsurface environment includes addition of suitable food source and electron acceptor/donor to the nitrate contaminated groundwater. Another approach may be to pass the contaminated groundwater stream through a 'reactive barrier' capable of mitigating nitrate contamination. Such a barrier may be placed across the groundwater flow, such that the nitrate concentration in groundwater is reduced as the water passes through the barrier.

2.5.1 Physico-Chemical Methods

Conventional physico-chemical methods for nitrate removal include selective ion exchange, reverse osmosis, and electrodialysis (Hoek et al. 1988). Murphy (1991) demonstrated chemical reduction of nitrate to ammonia and nitrogen using aluminium powder. The pH range of this reaction was 9-10.5. Ammonia released as above was removed from the system using air stripping or other ammonia removal methods. The above methods, though often quite efficient in nitrate removal, are relatively expensive to operate and hence uneconomical. Most of these treatment processes are also limited by the production of nitrate concentrated waste streams that may pose a disposal problem (Kapoor and Viraraghavan, 1997). Hence the interest in biological denitrification processes, which have been discussed in the next sections.

2.5.2 *Heterotrophic Denitrification*

Removal of nitrate from water using heterotrophic microorganisms is the most studied and widely used biological process for the denitrification of water. A wide variety of solid, liquid, and gaseous organic substrates have been studied as food source for such microorganisms. The most common substrates are methanol, ethanol, and acetic acid. Other less studied substrates include methane, carbon monoxide, and cellulose. A large body of work on this topic was conducted in 1980s in Europe, a review of which is presented below.

As reported by Richard et al. (1980), a pilot scale fluidized-bed reactor was operated using ethanol and/or acetic acid as the substrate and without effluent recycles. The system was modeled using Monod kinetics, considering carbon, nitrogen, and phosphorous as substrates. The 'maximum specific nitrate utilization rate' was reported to be 250 mg NO_3^- /h/g VSS. Actual denitrification rate, however, was strongly related to the flow velocity through the column, with denitrification rates being greater at lower velocities. The first commercial drinking water denitrification facility in France was constructed in 1983 in Eragny (Philipot et al. 1985). This 80m^3/h facility required ethanol and phosphate addition before passing the water through a biologically active clay filter. Post denitrification treatment included coagulant addition, activated-carbon/sand filtration, and disinfection. The nitrate concentration in the water was reduced from 68 mg/L to 25 mg/L, and nitrite concentration in the effluent was maintained at less than 0.05 mg/L. Denitrification in up-flow fixed-bed reactor was reported from Chateau London, France in 1983 (Frick and Richard, 1985). This 50 m^3/h facility required acetic acid and phosphate addition for denitrification, followed by coagulant addition, activated carbon filtration, and chlorination. Nitrate concentration in water was reduced from 80 mg/L to 30 mg/L. Nitrite accumulated initially after start-up but decreased to less than 0.1 mg/L after process equilibrium was achieved. A large number of fixed-film denitrification processes similar to ones described above were reported to be in operation in France using methanol, ethanol, or acetic acid as substrates (Gayle et al., 1989). A similar German study (Sontheimer et al. 1987) on denitrification evaluated post-treatment using $FeCl_3$ to reduce effluent nitrite concentrations following fixed-film denitrification using

acetic acid. Other German contributions include a fixed-film biological denitrification process using ethanol (Roennefahrt, 1985), which was commercialized under the trade name Denipor. The process consisted of ethanol and phosphate addition, a fixed-film filter packed with floating 'Styropor' spheres, aerobic biological filtration, and chlorination. 95% nitrate removal efficiency was obtained at a filter-loading rate of 1.0 kg NO_3^-/m^3 per day and at re-circulation rate of 200-500%. But, water quality was not improved by additional post-treatment with ozone and activated carbon or coagulants. Frank and Dott (1985) reported on the performance of a pilot-scale bioreactor packed with polystyrene beads, using methanol or ethanol as an energy source. Reduction of nitrate concentration from 55 mg/L to 3 mg/L was reported during this study. Nilsson and Ohlson (1982) studied denitrification in a series of bench-scale columns, packed with immobilized *Pseudomonas denitrificans* cells. The bacteria were encapsulated in a sodium alginate polymer and ethanol was used as a carbon source. Using four columns in series, the nitrate concentration was reduced from 104 mg/L to 0.1 mg/L. Nitrite accumulated in the effluent from the first three columns, but was reduced to 0.3 mg/L in the effluent from the fourth column. Yull-Rhee and Futts (1978) studied denitrification with methane, using two bench-scale sand columns in series. The first column was seeded with *Methylomonas* species and was purged with methane and air. The *Methylomonas* oxidized methane under aerobic conditions but did not denitrify. The second column was seeded with *Pseudomonas stutzeri* and supplied with the effluent of the first column but with no additional carbon source or air. The *Pseudomonas* was shown to denitrify using the metabolites produced from methane as carbon and energy source, resulting in symbiotic relationship between organisms representing two different trophic groups. Krantzenstein (1982) used methane for denitrification in three-stage process consisting of oxygen removal, denitrification in a biological filter, and re-aeration. Fuchs (1985) received the patent for a denitrification process using carbon monoxide saturated flexible porous carriers. When depleted, the carriers were resaturated with CO and recycled back to biological reactor. Rauschmaier and Barotke (1985) evaluated 'Birch-wood' as a substrate for denitrification. Bacteria used the cellulose in 'Birch-wood' readily and denitrification was achieved. However lignin tended to accumulate. Bullermann and Keidel (1986) evaluated whey as a carbon source for

denitrification in a fluidized-bed reactor. Nitrate reduction was achieved, but the denitrification rate was slow. A hybrid ion exchange/biological-denitrification process was studied by van der Hoek and Klapwijk (1988). This process utilized ion exchange for the removal of nitrate from ground water and incorporated biological denitrification as part of the resin-regeneration step. During regeneration a concentrated NaCl (10-15 g/L) or $NaHCO_3$ (25-30 g/L) solution was circulated in a closed loop between the ion-exchange column and an up-flow sludge bed (USB) denitrification reactor. Methanol was used as a substrate for the USB. The strong brine solution regenerated the exchange resin and the USB removed the nitrate (700 mg NO_3^--N/L) that accumulated in the brine. Removal of the nitrate allowed for reuse of the brine through several regeneration cycles. A sand filter was used in the loop to minimize carryover of biological solids and organic matter to the exchange resin. After regeneration the exchange resin was disinfected. However, sulfate can interfere with nitrate removal since most anion exchange resins are more selective for sulfate than nitrate.

Regarding rates of denitrification, several authors (Balderston et al., 1976;Hartnett, Ingersoll and Baker, 1999) have noticed that the higher the C:N ratio, the quicker the nitrate removal rate. However, even if excess carbon furthers the denitrification rate, it could bring about a quick decrease of oxidation-reduction potential, which enhances sulfate reduction, releasing toxic sulfides in an environmental setting.

The review of the heterotrophic denitrification process as presented above, indicate that such processes were generally able to reduce nitrate concentration in water to below the specified standards. However, some drawbacks were also noticed, as mentioned below.

- Growth of heterotrophic bacteria is limited by the source of organic carbon. Arrangement for the supply of organic nutrient (methanol, ethanol, acetic acid etc) is always a problem.
- Heterotrophic denitrification generally produces excessive biomass and soluble microbial products that require subsequent treatment.

- Denitrification using methane or carbon monoxide is still required to be studied further and their denitrification rate is not impressive either.
- The post treatment operations following the denitrification are not always very effective.
- Operation and maintenance of denitrification plants was found to be expensive.

2.5.3 Autotrophic Denitrification

The autotrophic denitrification process does not require organic carbon as food or energy source. However, inorganic carbon, i.e., bicarbonate/carbonate, must be provided as food source. In addition, a suitable electron acceptor must also be provided. Although autotrophic denitrifying bacteria are generally expected to function in anaerobic conditions, in many cases autotrophic mode of denitrification have been shown by facultative organisms i.e. *Paracoccus denitrificans, Thiobacillus denitrificans*, etc.

Hydrogenotrophic denitrifiers, i.e., organisms utilizing hydrogen as energy source, are ubiquitous in nature (Till et al., 1998, Gamble. 1976), which enhances the potential of denitrifying water using autotrophic means by using such microorganisms. Autotrophic denitrification in drinking water was studied by Kurt et al. (1987) using hydrogen in a bench-scale fluidized bed reactor. The process was modeled using a double-Monod saturation function. A commercial design for autotrophic denitrification called 'Denitropur' was introduced by Sulzer Water and Waste water Treatment (Hellekes. 1986), which incorporated indirect hydrogen saturation, phosphate addition, four packed bed reactors in series, post aeration, coagulant addition, filtration and UV disinfection. Carbon dioxide was added as an inorganic carbon source and to buffer against an alkaline pH shift. At the operating temperature of 10.5 °C, the microorganism growth rate varied from 0.1 to 0.3 per day. The sludge production was approximately 0.2 kg/kg nitrogen removed, on a dry weight basis. Residence time of 1 to 2 hours was required to remove 50 mg/L nitrate. The denitrification rate in the system varied with mass and activity of the biomass. In another study, *Alcaligenes eutrophus*, a hydrogenotrophic denitrifier was immobilized in polyacrylamide and alginate copolymer to evaluate denitrification in fluidized-bed and as well as batch reactors, and to elucidate rate of autotrophic

denitrification for obtaining the appropriate operating conditions for drinking water treatment (Chang et al. 1999). The maximum rate of denitrification was found to be (0.6 -0.7 kg-N/m^3/day) in flow through system. Denitrification was limited by hydrogen concentration. Phosphate concentration also affected the denitrification rate in presence of nitrite. High initial nitrite concentration was observed in case of batch reactors.

Autotrophic denitrification with reduced sulfur as the energy source has also been studied in some detail. Ability of pyrites to act as electron donors for microbial reduction of nitrate in anoxic subsurface environment has been shown to be one of the prime reasons for nitrate reduction in aquifer (Postma et al., 1991). Overath et al. (1986) studied autotrophic denitrification using columns packed with elemental sulfur and activated carbon. The columns were 100 mm in diameter and 3 m long and were operated at a volumetric loading rate of 30 L/h. After 15 days of start-up period, influent nitrate concentrations were reduced from 35 mg/L to 0 mg/L. Denitrification was also studied in columns packed with various ratios of elemental sulfur and limestone marl by Blecon et al. (1983). Efficiency increased as the particle size of the packed material was decreased. Efficiency of autotrophic denitrification was studied in a bench scale completely mixed batch reactor using calcium alginate beads suspension. The beads were composed of elemental sulfur, calcium carbonate, and *thiobacillus denitrificans* encapsulated in the calcium alginate polymer. Mixing in the reactor was provided by compressed nitrogen gas. Nitrate was reduced from 27 mg/L to 6 mg/L in 7 hours. The initial nitrogen removal rate was 1.6 mg N/L-h and increased to 4.8 mg N/L-h after approximately 4 hours. Nitrite tended to accumulate initially but was later reduced to less than 2 mg/L. Various authors have evaluated the role of reduced-sulfur compounds such as sulfide and thiosulfate for the denitrification of water and domestic or industrial wastewater (Claus1985, La Motta Diaz 1985, Martin 1982, Batchelor et al., 1978). Sulfate was by-product of denitrification using all above reduced-sulfur compounds. It was noticed that in some cases, denitrification of industrial wastewaters containing very high nitrate concentration (up to 6,000 mg/L) resulted in high sulfate concentration and led to sulfate inhibition. In water treatment where nitrate concentrations are lower, sulfate inhibition would not be expected. Stoichiometrically, the denitrification of 152 mg/L of nitrate

using elemental sulfur would yield 250 mg/L sulfate. In such cases, the potability of the water may be affected adversely. Batchelor and Lawrence (1978) developed a mathematical model to describe denitrification kinetics using elemental sulfur as a substrate. Sulfur diffusion through the bio-film, nitrate diffusion through the bulk solution, and nitrate diffusion through the bio-film were considered as possible rate-limiting steps. The influence of each of these steps on the overall reaction rate varies as the concentration of nitrate in the bulk solution varies. A mathematical model was developed by LeCloirec et al. (1985) for denitrification kinetics by *Thiobacillus denitrificans* on a sulfur-calcium carbonate filter. The model considers biomass growth, nitrate removal, nitrite evolution, and consumption sulfur.

Autotrophic and heterotrophic denitrification in identical columns was compared (Vidal et al. 2002) to remove nitrate from a closed system. Autotrophic denitrification rate was found to be higher (20.6-39.8 moles/day) as compared to heterotrophic denitrification (9.9-11.2 moles/day). For the heterotrophic system, organic carbon was found to be the chief controller of the denitrification rate and was also responsible for maintaining anaerobic environment within the reactor. Similarly for the autotrophic system, inorganic carbon was found to be the important parameter. Formation of significant amount of ammonia was also reported in both studies.

Ammonia removal is often achieved using nitrification/denitrification systems. In such systems, nitrifying bacteria oxidize ammonia to nitrate under oxic condition, and nitrate is subsequently or simultaneously reduced to nitrogen gas, under anoxic conditions. Nitrifying bacteria are mainly autotrophic and derive energy from oxidation of ammonia or nitrite. The oxidized nitrogen compounds can be used as alternative electron acceptor by denitrifying bacteria. Sliekers et al. (2002), in their paper have described this concept of completely autotrophic process, in which aerobic ammonia oxidizers and anaerobic ammonia oxidizers simultaneously oxidize ammonia to nitrogen gas and a small amount of nitrate. This is achieved in one single reactor, at oxygen-limited conditions, without the production of N_2O or NO. This process has been called CANON (Completely Autotrophic Nitrogen-removal over Nitrite). Carvantes et al. (2001) studied

denitrification in UASB reactor using different nitrate loading rate, different C:N ratios, limited acetate and NH_4-N. Ammonium was used as an alternative electron donor in this study, as described in the case above.

Application of limestone to supply alkalinity for the control of pH in autotrophic denitrification reactors, along with the optimum ratio of limestone to sulfur has been studied (Liu et al. 2002). Soares (2002) has studied denitrification in packed bed column reactors using bicarbonate and elemental sulfur. Oh. et al. (2001) investigated the effects of organic compounds (methanol and landfill leachate) on sulfur utilizing denitrification. In this study, a number of elemental sulfur-containing columns were operated under autotrophic, mixotrophic, heterotrophic conditions for approximately 1 year. The performance of the column indicated that the mixotrophic column had a higher nitrate removal capacity than the purely autotrophic column. It was also reported that under mixotrophic conditions, some portion of nitrate was removed heterotrophically and sulfur-utilizing autotrophic bacteria denitrified remainder without inhibition by organics. Lee et al. (2001) had studied the effect of external carbon source and empty-bed-contact-time (EBCT) on simultaneous heterotrophic and sulfur-utilizing autotrophic denitrification. Flere et al. (1999) studied the feasibility of sulfur/limestone-based autotrophic denitrification (SLAD) in pond systems for in-situ remediation of nitrate-contaminated surface water. Under anoxic conditions some encouraging results were obtained. Zhang et al., (1998) studied the SLAD process in batch reactors. The feasibility of the process was demonstrated under both oxic and anoxic environment. Kerri et al. (1998) evaluated two cathode materials and the impact of copper on bio-electrochemical denitrification. Koenig et al. (2001) worked on the kinetic model of autotrophic denitrification in sulfur packed-bed reactors. Autotrophic denitrification of synthetic wastewater by *Thiobacillus denitrificans* in upflow sulfur packed-bed reactors was studied there in order to establish process kinetics for prediction of effluent concentration. Qu et al. (2002) demonstrated a combined two-step process of heterotrophic and electrochemical autotrophic denitrification to treat nitrate contaminated drinking water. Performances were studied under different C:N ratios. Qu et al. (2001)

reported autotrophic denitrification of groundwater using electrochemical reactions (activated carbon fibre electrode) to produce hydrogen.

As can be seen from the discussion presented above, various researchers have tried different approaches for enhancement and improvement of autotrophic denitrification reactions for nitrate removal. Based on these discussions, it may be concluded that despite considerable research in this field, and encouraging results, some problems associated with the autotrophic denitrification process remain. These include,

- Availability of hydrogen for hydrogenotrophic denitrification. As per theoretical calculations, stoichiometrically, 0.35 mg/L of H_2 is required for complete denitrification of 1.0 mg/L NO_3-N. Provision of this hydrogen is costly and difficult.
- The autotrophic denitrification process is most effective when the pH is maintained near neutral. However pH of the system tends to increase in case of hydrogenotrophic denitrification, and tends to decrease in case of sulfur assisted systems. Hence buffering of the system is very important for ensuring optimal autotrophic denitrification rates.
- Accumulation of nitrite is sometimes observed during autotrophic denitrification, particularly in batch reactors (Vidal et al. 2002).
- Sulfur based system always have potential danger of sulfate or sulfide toxicity.

2.5.4 Denitrification in Natural Subsurface Environment

Number of attempts has been made to identify and quantify biological denitrification in natural subsurface environment. Vogel et al. (1980) reported a process of very slow denitrification in a confined aquifer by investigating the nitrate, oxygen, nitrogen and argon concentrations and $^{15}N/^{14}N$ ratios in artesian groundwater with radiocarbon ages ranging up to 27,000 years. Denitrification in sandy aquifer was also observed by Trudell et al. (1986) by measuring nitrate concentration below the water table. Denitrification was assayed by the acetylene blockage technique in slurried core material obtained from a fresh water sand and gravel aquifer by Smith et al. (1988).

2.6 Inspiration for the Present Study

As mentioned earlier in this chapter, one of the main hindrances in the widespread adaptation of hydrogenotrophic autotrophic denitrification for environmental engineering applications is the high cost and technical difficulties in the supply and production of hydrogen gas. In this regard, anaerobic corrosion metallic iron for in-situ production of hydrogen has for the above process seems to be a promising option. This is specially so because metallic iron is relatively inexpensive and non-toxic in low concentrations.

Metallic or zero-valent iron, Fe (0) is inherently unstable in both aerobic and anaerobic environments. In the presence of oxygen, Fe (0) is oxidized slowly to Fe (II) and Fe (III) by the process commonly known as rusting as shown by equations 2.7 and 2.8 below.

Aerobic Iron Corrosion: $$2Fe(0)+O_2+2H_2O \rightarrow 2Fe^{2+}+4OH^- \quad (2.7)$$

$$4Fe^{2+}+O_2+10H_2O \rightarrow 4Fe(OH)_3(s)+8H^+ \quad (2.8)$$

Fe (0) may also be oxidized in anaerobic environments, resulting in the reduction of water to hydrogen gas, as shown by equation 2.9 below.

Anaerobic Iron Corrosion: $$Fe(0)+2H_2O \rightarrow H_2+Fe^{2+}+2OH^- \quad (2.9)$$

Under the circumstances it is conceivable that corrosion of Fe (0) in anaerobic environments may supply hydrogen that is required for autotrophic denitrification of nitrate as per equation 2.4 mentioned earlier, resulting in a combined reaction as described by equation 2.10.

Biotic Nitrate Reduction: $$5Fe(0)+2NO_3^-+6H_2O \rightarrow 5Fe^{2+}+N_2+12OH^- \quad (2.10)$$

The above idea was tested by Till et al. (1998). Based on the results of this study, it was concluded that autotrophic denitrification of nitrate by hydrogen gas produced from iron corrosion is possible using both pure culture (*Paracoccus denitrificans*) and a mixed culture of autotrophic denitrifying microorganisms. However, certain additional questions were raised during this study, adequate resolution of which will ensure that Fe (0)-assisted autotrophic denitrification may be practically feasible. These points were as follows,

- There is a possibility of abiotic reduction of nitrate through direct reaction with Fe (0), as shown by equation 2.11 below.

Abiotic Nitrate Reduction: $4Fe(0) + NO_3^- + 7H_2O \rightarrow 4Fe^{2+} + NH_4^+ + 10OH^-$ (2.11)

Hence, end-product distribution of the nitrate removal process may be adversely affected, i.e., more ammonia formed and less nitrogen gas generated, if the abiotic nitrate removal process gains precedence. Proper reaction conditions must be determined to prevent this occurrence.

- Both abiotic and microbe assisted denitrification processes, equations 2.10 and 2.11 above, result in the production of OH^- ions, which may elevate the pH of the system. Hence, proper buffering must be provided such that pH increase is not excessive and detrimental to the nitrate reduction reactions.

The prime objective of the study described in this dissertation is to examine some of the above questions in detail, and thus answer the current unanswered questions regarding practical feasibility of Fe (0)-assisted autotrophic denitrification for various environmental engineering applications.

CHAPTER III
SCOPE AND OBJECTIVES

As mentioned in the previous chapter, the possibility of Fe (0)-assisted autotrophic denitrification, i.e., denitrification using hydrogen generated by the anaerobic corrosion of zero-oxidation state iron, Fe (0), as the energy source, inorganic carbon as food source, and nitrate as the terminal electron acceptor has already been demonstrated. The process holds great potential for the use in 'in-situ' reactive barriers containing iron metal and autotrophic denitrifying organisms for remediation of nitrate contaminated groundwater resources. This is due to the relatively low cost and non-toxicity of iron metal (in low concentration), and relative rapidity, low sludge production and non-requirement of organic substrate for the autotrophic denitrification process, as compared to heterotrophic denitrification process. Conceivably, such barriers can be placed around tube-well clusters supplying water for human consumption.

However, before such barriers become practically feasible, certain unresolved questions regarding such barriers needs to be addressed. They include, among other things, types of iron metal that may be suitable, rates of biological denitrification, microbial inhibition due to pH increase, formation of ammonia due to abiotic reduction of nitrate in the presence of Fe (0) in anoxic conditions, and long-term performance of such barriers. Related research reports suggest that in a combined Fe (0)-microbial treatment system for denitrification, the rate of Fe (0) oxidation depends on the type and specific surface area of iron metal used. Also, the abiotic and biological processes for nitrate reduction represent competing pathways, with the abiotic pathway out-competing the biological process if the Fe (0) surface area is high.

The present research is geared towards answering questions regarding type and surface area of iron metal required in effecting a favorable end-product distribution, i.e., formation of nitrogen gas as opposed to ammonia as the primary by-product of the nitrate reduction by Fe (0) assisted denitrification. Accordingly, two parameters, whose effect on biological denitrification will be studied in detail are, the type of metallic iron used,

and Fe (0) surface area. In addition, some preliminary experiments concerning sustenance of biological denitrification through externally supplied hydrogen, either directly supplied or produced in-situ by anoxic corrosion of Fe (0) will also be conducted. It is expected that based on this and other related studies, optimal composition for reactive barriers capable of providing conditions for maximizing long-term biological denitrification will be established.

Specifically, the objectives of this dissertation are:

- Developing and maintaining a mixed culture of hydrogenotrophic denitrifying microorganisms in the laboratory.
- Demonstration of the ability of the microorganisms cultured as above to sustain autotrophic denitrification reactions with external hydrogen supply.
- Demonstration of the ability of the same microorganisms to sustain autotrophic denitrification reactions with hydrogen derived from anoxic corrosion of Fe (0) in aqueous media.
- Characterization of Fe (0) obtained from different sources in terms of abiotic nitrate-reduction capacity, and resulting ammonia formation. This will result in the selection of the appropriate type of Fe (0) for biological denitrification applications.
- Determination of the effect of iron-surface area on extent of denitrification and end product distribution determined as a function of time of interaction in batch reactors.
- Determination of the effect of iron-surface area on extent of denitrification and end product distribution determined as a function of retention time in flow through column reactors.
- Analysis and discussion of results, resulting in conclusions regarding optimal composition of material for effecting 'in-situ' hydrogenotrophic denitrification with favorable end-product distribution.
- Identification of further research needs in the area, based on the conclusions drawn from the results presented in this dissertation.

CHAPTER IV

ANALYTICAL METHODS AND EXPERIMENTAL PROCEDURES

4.1 Introduction

Analytical methods used for the research described in this dissertation have been discussed in some detail in this chapter. In addition, information, including make and model number of various instruments used for various analyses have also been mentioned. This is followed by a detailed description of the experimental setups used for conducting the experiments, and the procedure followed for conducting the experiments using the experimental setups mentioned above.

4.2 Analytical Methods

In this section, a description of the chemicals, glassware, chemical stock solutions, instruments and analytical methods used for the experiments described in this dissertation is provided. Mostly, proven analytical methods were used for measuring various parameters. Wherever non-standard methods are used, full description of the method is provided.

4.2.1 Chemicals and Glassware

Reagent grade chemicals were used for preparing mineral medium required for bacterial culture development, and for various experiments described in this dissertation. Analytical grade chemicals were used for preparing the eluent and various standards for ion chromatographic determinations of nitrate and nitrite. All glassware used in the experiments was of 'Borosil' or 'Corning' brand and were thoroughly cleaned to prevent interference and contamination. Hydrogen and nitrogen gas of Iolar grade was used for developing and maintaining bacterial culture and other experiments.

4.2.2 Preparation of Stock Solutions

All stock solutions and standards were made with ordinary distilled or triple distilled water. Triple distilled water, or Milli-Q water was also used for most experiments and analyses described in this research.

4.2.3 Measurement of Nitrate and Nitrite

Nitrate and Nitrite were measured using an Ion Chromatograph (Metrohm 761) equipped with a Phenomenex STAR ION A 300 IC anion column, and conductivity detector with ion suppression. The method used (Method No. 2.6.3) for this purpose was specified in the relevant company literature. All samples for nitrate/nitrite determination were filtered through 0.2 μm filter paper before injection into Ion Chromatograph.

4.2.4 Ammonia Measurement

Ammonia was measured colorimetrically by Nesslerization (Method No. 417 B, APHA, et al., 1985). A spectophotometer (Spectronic, 20 D^+, India) with Borosil glass absorbance cells having 1 cm path length were used for this purpose.

4.2.5 Iron Measurement

Iron (II) was analyzed using 1,10 phenanthroline method as described in Standard Methods (APHA et al., 1985). Total iron estimation was carried out using an Atomic Absorption Spectrophotometer (spectrAA-20, Varian). Samples were acidified with concentrated HNO_3 before such estimation. Iron standards were prepared by dissolving reagent grade $FeCl_3$ in distilled water acidified with concentrated HNO_3.

4.2.6 pH Determination

pH was measured using a combination pH electrode (Toshniwal CL-51, India) connected to a digital pH meter (Toshniwal CL-54, India). pH was also checked using pH paper (range pH: 2-10.5) when sample volume was less.

4.3 Experimental Procedures

Various types of experiments were conducted such that the research objectives of this dissertation as outlined in Chapter III could be realized. The need for maintaining sterile blanks for highlighting microbial activity was realized and hence sufficient numbers of blanks were run along with every type of microbiological experiment. Sufficient care was taken to maintain sterile conditions wherever the experimental protocol so demanded.

4.3.1 Preliminary Experiments: Development and Maintenance of Bacterial Culture

A mixed culture of autotrophic denitrifying microorganisms was developed and maintained in a glass reactor as shown in Figure 4.1. Initially, 1000 mL of a mineral medium prepared with sterilized distilled water, and containing inorganic carbon (HCO_3^-), substrate (NO_3^-), buffer ($H_2PO_4^-$) and other trace nutrients as per Table 4.1, along with 10 mL of wastewater treated by an anaerobic treatment process was fed to the

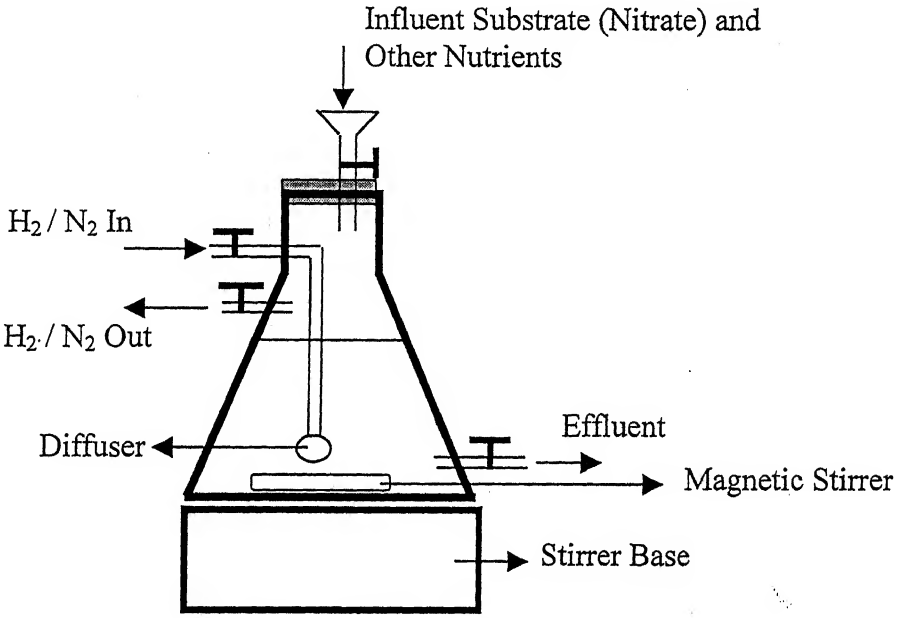

Figure 4.1 Schematic of the Apparatus Used for Developing and Maintaining the Mixed Culture of Autotrophic Denitrifying Microorganisms

reactor through the funnel at the top of the reactor. Next, this inlet was closed and the gas inlet and outlet to the reactor were opened. Reactor contents were purged with nitrogen gas for 10 minutes to ensure that oxygen was absent in the reactor. Then, hydrogen gas was fed to the reactor by the same procedure for 5 minutes, such that considerable amount of hydrogen gas can be present in the reactor. After this, the gas inlet and outlet ports were closed and the reactor contents were kept in continuously mixed condition by employing a magnetic stirrer. After every six days, reactor contents were again purged with hydrogen. While purging, 100 mL of reactor contents were withdrawn from the outlet at the bottom of reactor, and 100 mL of the mineral medium containing

Table 4.1 Composition of the Mineral Medium (adapted from, Till et al. 1998)

Nutrient	Concentration mg/L
$NaNO_3$	40 (as N)
$NaHCO_3$	250
KH_2PO_4	250
$CuCl_2.2H_2O$	0.0392
$ZnCl_2$	0.1363
$NiCl_2$	0.013
$FeCl_2.4H_2O$	0.7016
$AlCl_3$	0.1106
$MnCl_2.4H_2O$	0.2807
$CoCl_2.6H_2O$	0.0382
$Na_2MoO_4.2H_2O$	0.0254
H_3BO_4	0.0382
Na_2SO_4	0.1420

nitrate introduced from the top of the reactor. This corresponded to a mean cell residence time of 60 days for the microorganisms being cultured in the reactor. The effluent collected as above was analyzed for $NO_3^- - N, NO_2^- - N, NH_3 - N$, pH and absorbance at 600 nm, using 4 cm path length spectroscopic cell (Till et al. 1998). After bacterial growth was established, $NO_3^- - N, NO_2^- - N, NH_3 - N$ concentrations in the effluent, as measured every six days, were nearly zero, while the pH stabilized around 8.5. The absorbance value increased steadily and stabilized, indicating growth of microorganisms and subsequent maintenance of steady state microbial concentration in the reactor. Next, a second reactor was prepared in similar way as before, but without anaerobic effluent as seed. This reactor was instead seeded with 10 mL of the fully mixed contents of the first reactor. Once steady-state condition was obtained in the second reactor, a third reactor was started with seed from the second reactor. However the mineral medium in this case

was prepared with sterilized ground water from IIT Kanpur campus instead of distilled water. Finally, a fourth reactor was started with mineral medium made with sterilized ground water from IIT Kanpur campus and seed from the third reactor. This fourth reactor provided seed for all experiments described in the next sections. The reason behind adopting this procedure for preparing the stock bacterial culture solution was to ensure that a mixed culture of purely autotrophic denitrifying microorganisms would be isolated by providing the enrichment medium unique to this group of microorganisms (Smith et al. 1994). Replacement of distilled water by ground water in preparation of the mineral medium ensured that these microorganisms were acclimatized to the groundwater environment, and hence could be used in experiments involving groundwater.

4.3.2 Experiment Type I: Autotrophic Denitrification by Externally Supplied Hydrogen Gas

Experiment type I was carried out in bottles that contained the mineral medium similar to that specified in Table 4.1, but with varying nitrate concentrations. These bottles were seeded with microorganisms from the stock culture reactors described earlier. A typical bottle used for such experiments is shown in Figure 4.2. Volume of each bottle was 250 mL. At the start of the experiment, each bottle was filled with 200 mL of mineral medium prepared with sterilized distilled water. Nitrate concentrations in the six bottles were 40, 80, 120, 160, 200 and 240 mg/L (as N) respectively. In addition, a seventh bottle was prepared with nitrate (as N) concentration of 40 mg/L and was used as a blank. Next, all these reactors were sterilized. Then, 5 mL of seed from the stock culture was added to each of the six bottles. No seed was added to blank reactor bottle. Then the stopcocks on the two tubes of each reactor bottle were opened (see Figure 4.2) and the contents of each bottle were purged with nitrogen gas for 10 minutes so that any residual oxygen in the solutions was removed. Next, hydrogen was passed through each bottle for 5 minutes such that a considerable amount of hydrogen can be present in the mineral medium contained in each bottle. Hydrogen gas was applied to each bottle every 3 days, to ensure that the denitrification reaction in these bottles was never hydrogen limited. Samples were collected from each bottle in every 7-10 days.

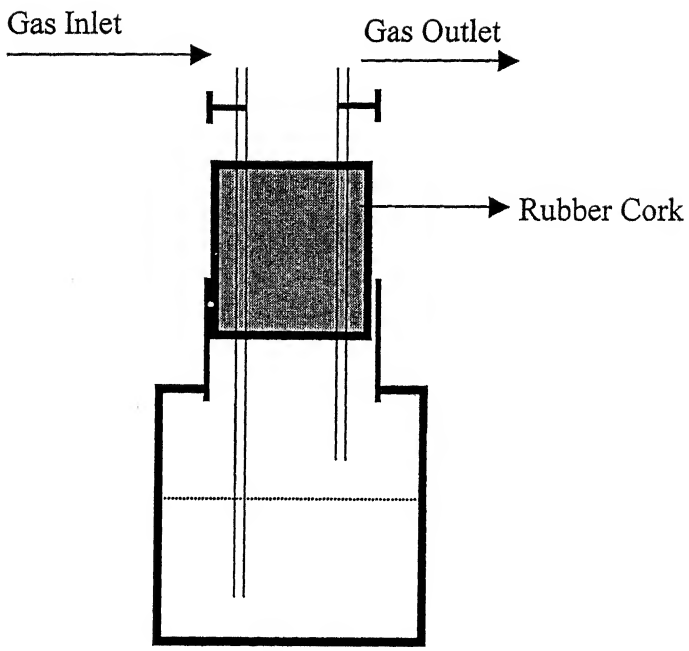

Figure 4.2 Typical Bottle Used for Type I Experiment

Sample collection involved opening the gas inlet and outlet tubes and applying hydrogen gas pressure through the tube that is not immersed in water. This ensured that liquid sample was ejected from the tube immersed in water. 20-25 mL of sample was collected from each bottle during each sample collection cycle. The collected samples were sterilized to ensure cessation of all bacterial activity, and stored for measurement of residual $NO_3^- - N, NO_2^- - N, NH_3 - N$, and pH.

4.3.3 Experiment Type II: Autotrophic Denitrification by Hydrogen Gas Generated from Anoxic Corrosion of Iron Metal

For the experiments of this type, hydrogen gas was generated by anaerobic corrosion of iron metal in a reactor of 1000 mL volume. The gas thus generated was conveyed through a manifold to seven reactors of 250 mL volume each containing various concentrations of nitrate. Of the seven reactors, six containing 40, 80, 120, 160, 200 and 300 mg/l (as N) nitrate respectively were seeded with autotrophic denitrifying microorganisms, while the seventh reactor containing 40 mg/L (as N) nitrate was not seeded.

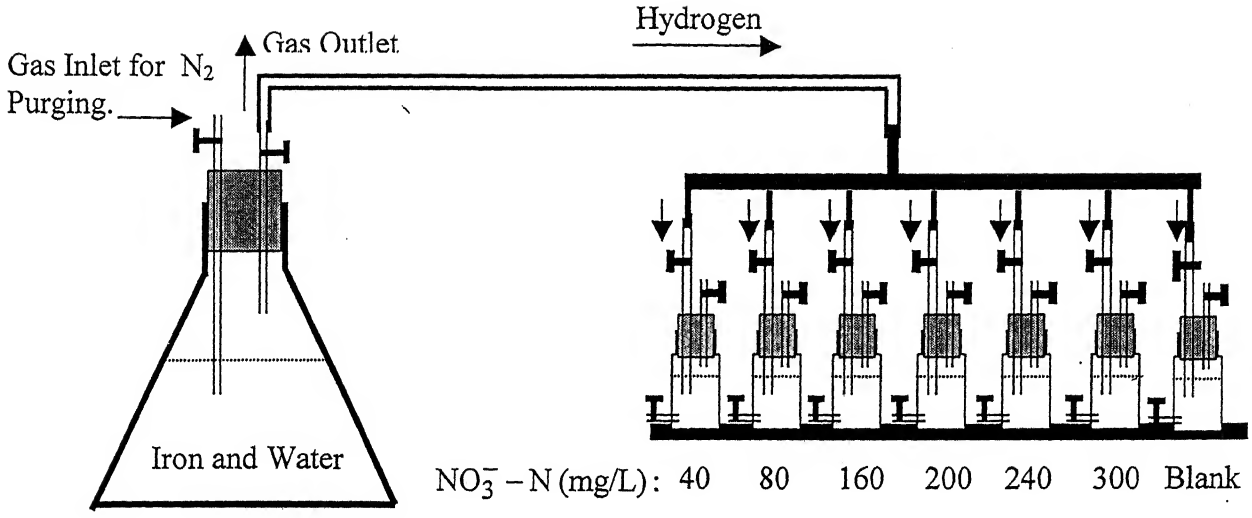

Figure 4.3 Schematic of the Experimental Setup used for Type II Experiment

A schematic of the experimental setup is shown in Figure 4.3. The autotrophic denitrification reactions took place in six 250 mL volume reactors with the help of hydrogen generated in the former reactor. To start the experiment, 10 g of electrolytic iron power and 1 liter of sterilized distilled water was added to the hydrogen generation reactor. Next, mineral medium of around 200 ml as specified in Table 4.1, prepared with sterilized IIT Kanpur groundwater with different initial nitrate concentrations i.e. 40, 80, 120, 160, 200 and 300 mg/L (as N) respectively, were fed to different seeded reactors. Blank reactor was fed with 40 mg/L nitrate (as N). The whole system was then sterilized. Next, contents of all the reactors, i.e., hydrogen generating reactor, seeded reactors and blank reactor were purged with nitrogen gas for 15 minutes to remove all residual oxygen in the system. Then the valves in the reactors were maneuvered in such a way that the hydrogen generated in the hydrogen-generating reactor can flow freely to seven other reactors without any interference from outside the system. The system was kept in this anoxic and abiotic state for 7 days to ensure that sufficient amount of hydrogen has migrated into the denitrification reactors to sustain a population of denitrifying microorganisms. After 7 days, 5 mL of seed taken from the stock culture reactors described earlier was introduced into the seeded reactors through the gas outlet tube.

Contents of the reactors were then mixed to ensure even distribution of microorganisms throughout the reactor. Samples were extracted from each denitrification reactor every 7-10 days by opening the sampling valve at the bottom of the reactor, whence sample flowed out automatically due to pressure generated inside the reactor due to hydrogen production and migration. 20-25 mL samples were collected from each reactor during each sample collection cycle; then the samples were sterilized and kept in storage for future analysis. Parameters analyzed were residual $NO_3^- - N, NO_2^- - N, NH_3 - N$, and pH.

4.3.4 *Experiment Type III: Iron Metal Mediated Abiotic and Biological Reduction of Nitrate under Anoxic Conditions in Batch Reactor*

These experiments were conducted in BOD bottles. A typical abiotic nitrate reduction experiment, i.e., without microbial intervention, consisted of filling a BOD bottle with mineral medium made with sterilized IIT Kanpur groundwater. The nitrate concentration in such a bottle was 40 mg/l (as N). Next, a predetermined quantity of iron metal was added to the bottle, after which the bottle was sealed and sterilized again. After the specified time period of reaction, which varied from 7 to 14 days, the bottle was unsealed and a sample extracted and sterilized before being stored for analysis of $NO_3^- - N, NO_2^- - N, NH_3 - N$ and pH. A listing of various experiments done in this fashion is given in Table 4.2.

A typical biological denitrification experiment consisted of filling a BOD bottle with mineral medium made with sterilized IIT Kanpur groundwater, but no nitrate. Next, a predetermined quantity of iron metal was added to the bottle, after which the bottle was sealed and sterilized. The bottle was left in sealed condition for 7 days to ensure that enough hydrogen has evolved through anoxic corrosion of iron metal to support microbial reactions. Next, a predetermined quantity of nitrate was added to the bottle such that nitrate concentration was 40 mg/L (as N). Also, 5 mL of seed from the stock culture reactor described earlier was added to the bottle. The bottle was then resealed and

the contents well mixed. After the specified time period of reaction, which varied from 7 to 60 days, the samples were extracted and sterilized before being stored for analyzing $NO_3^- - N, NO_2^- - N, NH_3 - N$, and pH. A listing of various experiments done in this fashion is given in Table 4.3.

Table 4.2 List of Abiotic Iron Metal Assisted Nitrate Reduction Experiments Conducted in Batch Reactors

Sl. No.	Iron Type	Iron Concentration (g)	Initial Nitrate Concentration (mg/L)	Reaction Type	Reaction Duration (Days)
1.	Iron Powder[1]	1	40	Abiotic	2, 4 and 6
2.	Iron Filing[2]	1	40	Abiotic	2, 4 and 6
3.	Iron Shaving[3]	1	40	Abiotic	2, 4 and 6
4.	Steel Wool[4]	1	40	Abiotic	2, 4 and 6
5.	Steel Wool	2, 3, 4 and 5	40	Abiotic	7 and 14

Note:

1. Commercially Available: Batch No- 09003, Laboratory Reagent, Reidel-Chemicals (India).
2. Commercially Available: Batch No-111204, Laboratory Reagent, Nice Chemicals Pvt. Ltd.(India).
3. From IIT Kanpur Central Workshop
4. Commercially Available: Rohit Industries (India).

Table 4.3 List of Iron Metal Assisted Biological Denitrification Experiments Conducted in Batch Reactors

Sl. No.	Iron Type	Iron Concentration (g)	Initial Nitrate Concentration (mg/L)	Reaction Type	Reaction Duration (Days)
1.	Steel Wool	0.5	40	Biological	7, 14, 21, 30, 40 and 60
2.	Steel Wool	1.0	40	Biological	7, 14, 21, 30, 50 and 60
3.	Steel Wool	1.5	40	Biological	7, 14, 21, 30, 40 and 60

4.3.5 *Experiment Type IV: Iron Metal Mediated Abiotic and Biological Reduction of Nitrate under Anoxic Conditions in Intermittent-Flow Reactor*

These experiments were conducted in anoxic flow-through columns operated in the up-flow mode. Nitrate contaminated mineral medium was allowed to flow through columns containing sand and various amounts of metallic iron. The columns were kept sterile in case of abiotic experiments and were seeded with microorganisms from the stock culture in case of biological denitrification experiments. The columns were operated in the intermittent-flow mode so that sufficient retention time could be provided in the columns for nitrate reduction reactions to take place. Accordingly, samples were collected once a day for analysis.

The glass columns used for these experiments were approximately 12 cm in length and 4 cm in diameter. These columns were operated in the up-flow mode, with water influent from the bottom of the column. Each column consisted of two parts, a small inlet chamber and a main column with dimensions given above, separated by a porous sintered disk. Water influent to the column first entered the inlet chamber and then passed through the porous sintered disk to reach the main column, which contained the media. The sintered disk also acted as a support for the media in the main column above. The media in the column consisted of sand graded to 1-2 mm dia. Graded sand was then thoroughly washed with acid and water before drying in an oven. Next, the sand was mixed with varying quantities of steel wool, and loaded into the columns. Then the columns were sterilized. The height of media in each column was 10 cm, which corresponded to a media volume of 125 cm^3. Porosity of the media was approximately 50 percent. Each column was sealed at the top by a rubber stopper, with a glass tube passing though it for removing water effluent from the column.

The schematic of the experimental apparatus used in case of abiotic experiments is shown in Figure 4.4. Two types of media were used for these experiments, one containing only sand, and the other sand mixed with 1.0 g steel wool. At the start of the experiment, both

chambers of the two columns were fully flooded with freshly prepared warm distilled water, which contained little or no dissolved oxygen. The columns were then maintained in sealed condition for 7 days, to ensure onset of anaerobic conditions in the column. Next, the columns were connected to a reservoir containing mineral medium, as per specifications given in Table 4.1, and prepared with IIT Kanpur ground water. In addition to containing approximately 35 mg/L of nitrate (as N), 10 mg/L $HgCl_2$ was added to the mineral medium in the reservoir to inhibit any microbial activity. Then, the whole apparatus, including the reservoir, columns and connectors were sterilized to ensure complete cessation of all bacterial activity. Each column was then flushed with approximately 250 mL of mineral medium from the reservoir, so that the nitrate concentration in the effluent from the columns were approximately same as the initial

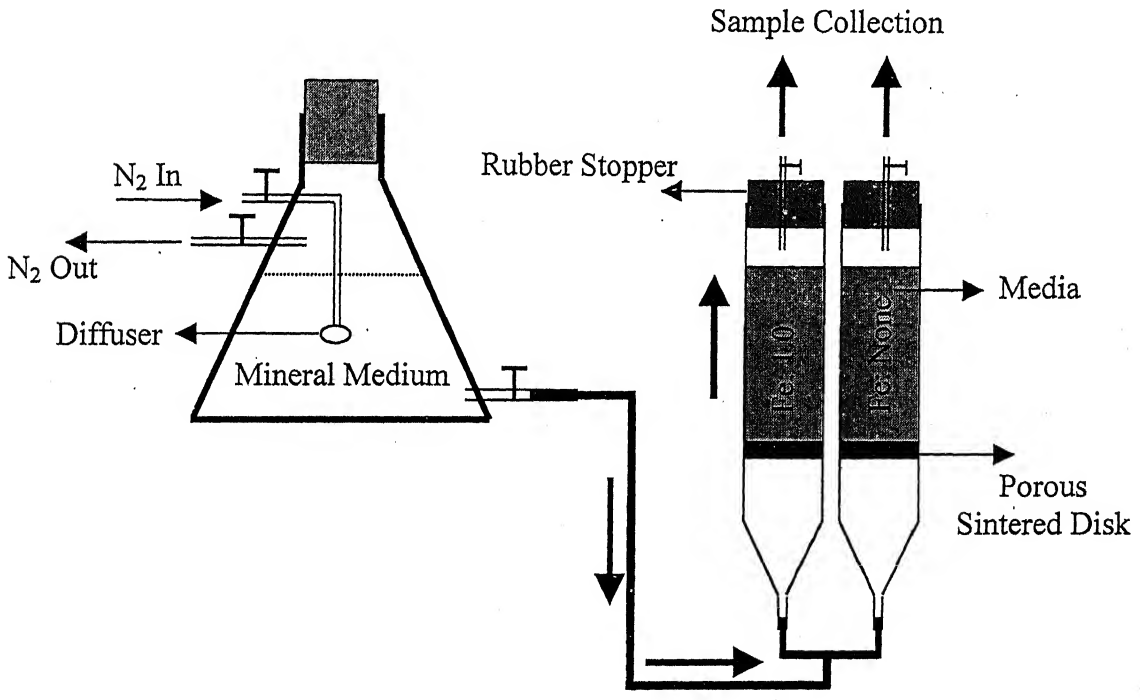

Figure 4.4 Schematic of the Experimental Apparatus used for Investigating Abiotic Nitrate Reduction in Flow-Through Columns

nitrate concentration in the reservoir. Then, the valves connecting the reservoir to the columns and those controlling water flow out of the column were closed, and the apparatus maintained in sealed condition. Once every 24 hours, the closed valves, as

described above, were opened and a fixed amount of effluent, either 50, 25, 10 or 5 mL corresponding to retention times of 1.3, 2.6, 6.5 or 13 days, was allowed to pass through the columns and collected. The collected samples were sterilized again, and stored for measurement of residual $NO_3^- - N, NO_2^- - N, NH_3 - N$, and pH.

In case of experiments involving biological denitrification, the up-flow columns as described earlier had to be seeded with microorganisms before the commencement of the actual experiment. The schematic of the apparatus used for seeding the columns is shown in Figure 4.5. The columns for this purpose were prepared as before with sand

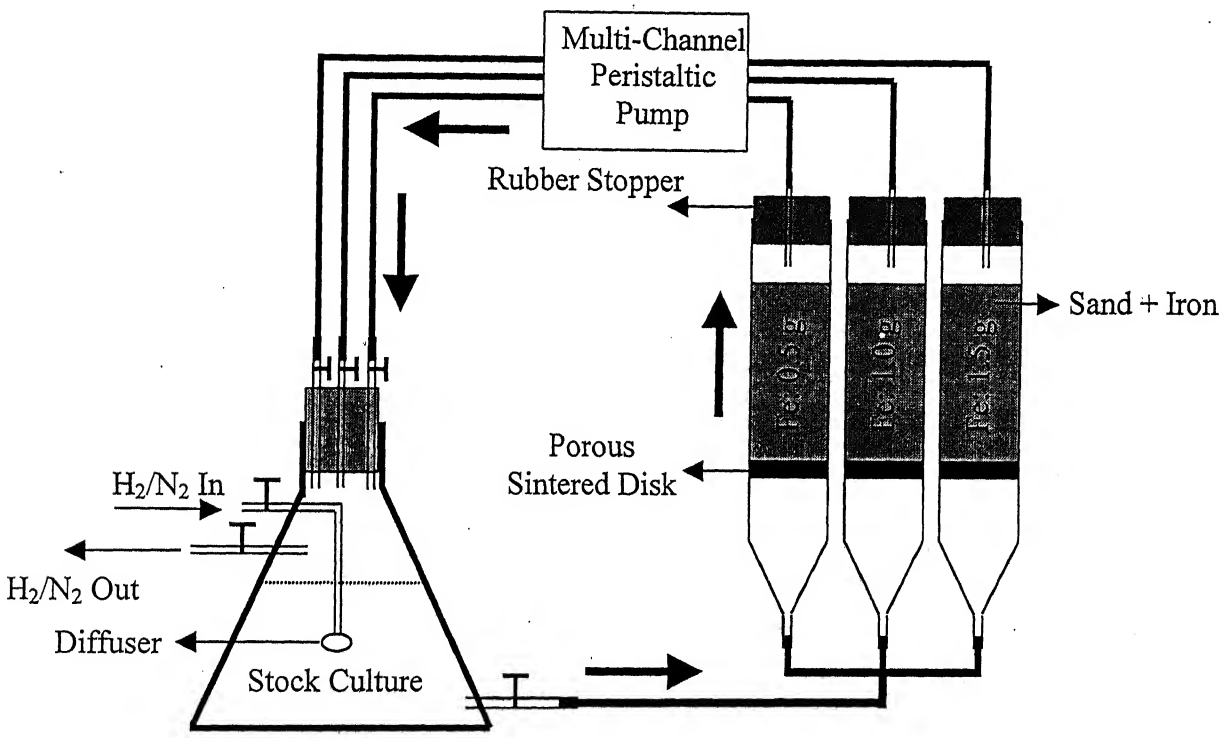

Figure 4.5 Schematic of the Apparatus Used for Promoting Attached Growth of Autotrophic Denitrifying Microorganisms in Up-Flow Columns

and varying metallic iron concentration as shown in Figure 4.5, and maintained as mentioned earlier, in flooded and sealed condition for some time to ensure the onset of anoxic conditions. Next the columns were connected to a stock culture reactor as shown in Figure 4.5. A multi-channel peristaltic pump was employed for re-circulating the

stock culture through the column. Initially the stock culture was periodically provided with hydrogen from an external source. However after some time, this supply of hydrogen was stopped. Colonization of the sand media in the columns by microorganisms was considered to have occurred once despite stopping external hydrogen supply, nitrate reduction was observed in the system. To further demonstrate this point, a reactor containing sterilized mineral media with 40 mg/L of nitrate (as N) was purged with nitrogen gas and attached to the columns in place of the stock culture reactor. It was observed that continued re-circulation of nitrate-containing mineral media through the reactor resulted in reduction in nitrate concentration in the system, even when no external hydrogen was supplied.

Biological denitrification experiments were carried out in an experimental setup similar to one shown in Figure 4.4. To start the biological denitrification experiments, the columns were connected to a reservoir containing a sterilized mineral medium prepared with IIT Kanpur tap water containing approximately 35 mg/L of nitrate (as N). Each column was then flushed with approximately 100 mL of the nitrate-containing mineral medium from the reservoir, so that the nitrate concentrations in the effluent from the columns were approximately the same as the initial nitrate concentration in the reservoir. After this, the valves connecting the reservoir to the columns and those controlling water flow out of the column were closed, and the apparatus maintained in sealed condition. Then as in the case of abiotic experiments described earlier, the closed valves were opened and a fixed amount of effluent was allowed to pass through the column and collected each day. The collected samples were sterilized, and stored for measurement of residual $NO_3^- - N, NO_2^- - N, NH_3 - N$, and pH.

CHAPTER V

RESULTS AND DISCUSSION

5.1 Introductory Remarks

As described in chapter II, section 2.6, in the hydrogenotrophic denitrification process, nitrate is the electron acceptor, which is ultimately reduced to nitrogen, thus resulting in denitrification. The denitrification process as described above, leads to the formation of a number of intermediate-products, and ultimately molecular nitrogen, as depicted below.

$$\overset{+5}{N}O_3^- \rightarrow \overset{+3}{N}O_2^- \rightarrow \overset{+2}{N}O(g) \rightarrow \overset{+1}{N}_2O(g) \rightarrow \overset{0}{N}_2(g) \qquad (5.1)$$

A mixed culture of hydrogenotrophic anaerobic denitrifying microorganisms capable of effecting denitrification as above was developed and maintained as described in Chapter IV, and used for the research described here. Hydrogen required for the sustenance of above denitrification process is either supplied externally or may be generated 'in-situ'. The 'in-situ' hydrogen generation technique studied in this research is through anaerobic corrosion of metallic iron in aqueous medium, as described by Equation 2.9. This is achieved by adding metallic iron to a system containing nitrate contaminated aqueous medium and hydrogenotrophic anaerobic denitrifying microorganisms. Addition of metallic iron in this manner also results in abiotic nitrate reduction, leading to the formation of nitrite and ultimately ammonia as shown below.

$$\overset{+5}{N}O_3^- \rightarrow \overset{+3}{N}O_2^- \rightarrow \overset{-3}{N}H_4^+ \qquad (5.2)$$

The ultimate objective of research described here is to determine the type and quantity of metallic iron to be added for biological denitrification. This determination is important so that ammonia formation by abiotic nitrate reduction (Eq. 5.2), which is undesirable, is kept to a minimum, while maintaining a reasonable rate of biological denitrification sustained by hydrogen production through metallic iron corrosion (Eq. 5.1).

Experiments of various types were carried out in a logical progression to achieve this goal. First, autotrophic denitrification supported by externally supplied hydrogen was demonstrated. Second, autotrophic denitrification supported by hydrogen generated through anaerobic corrosion of metallic iron was demonstrated. Third, the effect of iron

type on abiotic nitrate reduction was studied, and the type of iron exhibiting least propensity to abiotically reduce nitrate identified. The type of iron thus chosen was then used for all subsequent experiments. Fourth, the effect of iron surface area on ammonia production due to abiotic nitrate reduction was examined for both sterile (abiotic) and seeded (with denitrifying microorganisms) systems. Based on this study, appropriate iron surface area(s) were chosen for subsequent experimentation. Fifth, long-term denitrification experiments were conducted in batch reactors containing different quantities of iron, as determined through the previous experiment, to monitor the extent of nitrate removal, denitrification and ammonia formation. Finally, long-term denitrification experiments were conducted in up-flow column reactors containing a porous media, different quantities of iron, and denitrifying microorganisms to demonstrate the optimal conditions of biological denitrification process in such systems.

5.2 Autotrophic Denitrification with Externally Supplied Hydrogen

The first type of experiments sought to determine the ability of hydrogenotrophic anaerobic denitrifying microorganisms, as described above, to denitrify aqueous solutions contaminated with nitrate using externally supplied hydrogen as energy source. The experimental apparatus used, and methodology adopted for this purpose has been described in detail in section 4.3.2. As mentioned in that section, these experiments consisted of seeding bottles containing wide range of nitrate concentration, viz., 40, 80, 120, 160, 200 and 240 mg/L nitrate (as N) with the autotrophic denitrifying microorganisms and maintaining conditions rich in hydrogen through external hydrogen supply. Evolution of nitrate, nitrite and ammonia concentration was monitored with time in all reactors to determine the extent of denitrification. Similar monitoring in a reactor containing 40 mg/L nitrate (as N) but no added microorganisms, but supplied with hydrogen as in the other reactors showed no degradation of nitrate, or formation of nitrite and ammonia. Results of the experiments with seeded reactors are presented in Figure 5.1(A). As per data presented in this figure, nitrate was completely removed within 15 to 30 days in all reactors, with time required for complete nitrate removal increasing with increase in initial nitrate concentration in the reactor. Formation of nitrite as an intermediate-product of nitrate reduction was noticed in all reactors. Evolution of nitrite

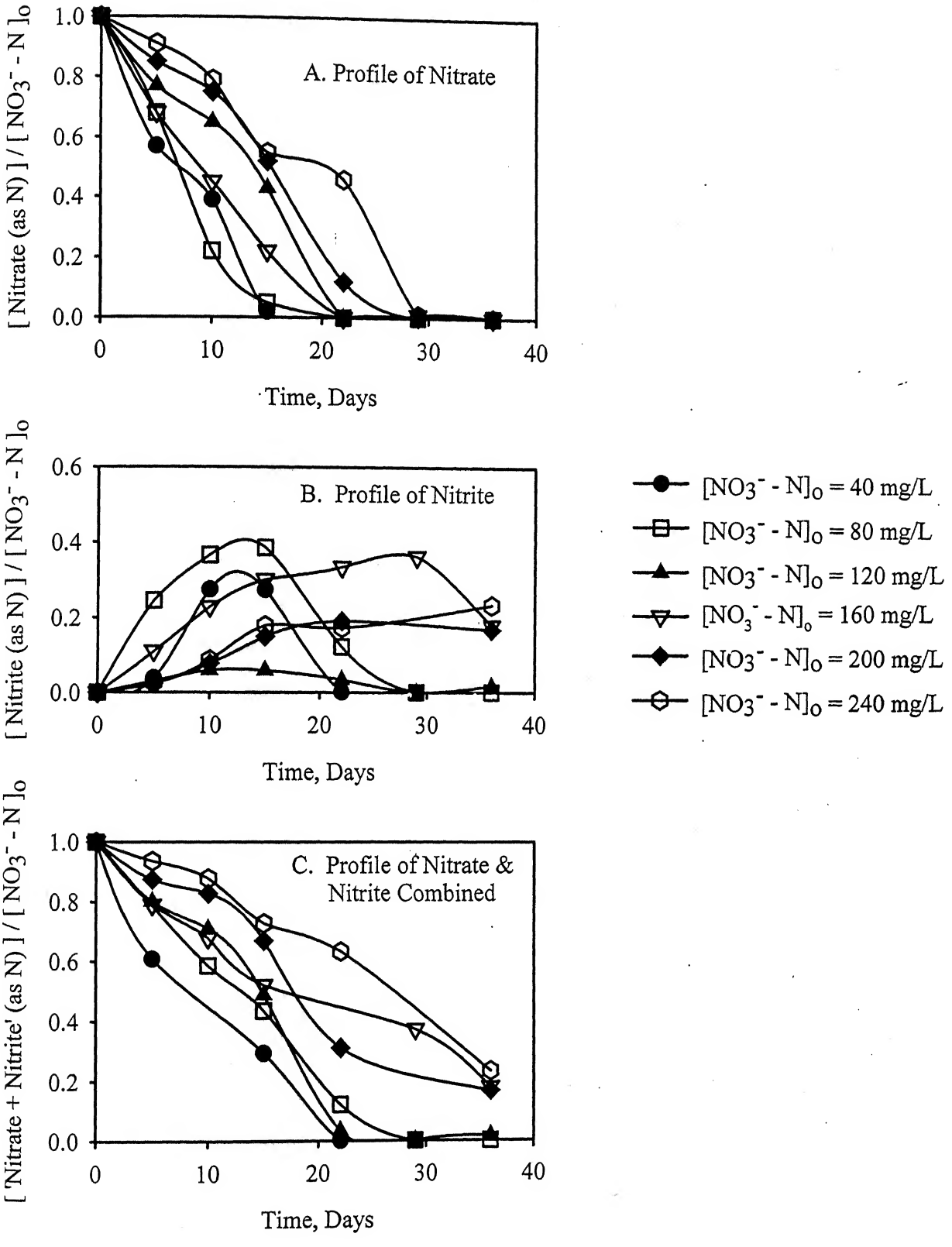

Figure 5.1 Reduction of Nitrate by Autotrophic Denitrification with External Hydrogen Supply
$[NO_3^- - N]_o$: Initial Nitrate Added

in all reactors is shown in Figure 5.1(B). As can be seen from this figure, nitrite concentration in all reactors initially increased with time to a maximum value of up to 40 percent of initial nitrate (as N) concentration, before decreasing with further passage of time. This suggests slower nitrite removal kinetics, as compared to nitrate removal rates, since nitrite is observed to persist in solution even after all nitrate is consumed. In addition, samples taken from the reactor were also tested for presence of ammonia. However, no ammonia could be detected in any of the samples tested. Thus, the extent of denitrification, i.e., removal of nitrogen from the aquatic matrix, can be determined from the evolution of total aquatic nitrogen, which is the sum of nitrate and nitrite nitrogen, with time, as presented in Figure 5.1(C). As per this figure, complete denitrification was observed in reactors with initial nitrate concentration of 40, 80 and 120 mg/L nitrate (as N) within 30 days. In reactors with higher nitrate concentrations, approximately 80 percent denitrification was observed up to the observation period of 35 days. Residual nitrogen species in these reactors after 30 days was entirely nitrite, which persists in solution longer due to its slower removal kinetics, nitrate having been completely removed from the system within 30 days. It is conceivable that this residual nitrite would also be removed if a reaction time longer that 35 days were provided.

5.3 Autotrophic Denitrification by Hydrogen Supplied through Anoxic Corrosion of Metallic Iron

The second type of experiments sought to determine the ability of hydrogenotrophic anaerobic denitrifying microorganisms to denitrify aqueous solutions contaminated with nitrate, using hydrogen generated through anaerobic corrosion of metallic iron (see Eq.: 2.9). The main difference between these and previous set of experiments was that the amount of hydrogen available in this case was expected to be much less than in the previous case, and hydrogen limiting conditions are expected to persist. The experimental apparatus used, and methodology adopted for this purpose has been described in detail in section 4.3.3. As mentioned in that section, these experiments consisted of connecting reactors containing 40, 80, 120, 160, 200 and 300 mg/L of nitrate (as N), and seeded with autotrophic denitrifying microorganisms, to a reactor containing iron powder and water in anoxic conditions. It was expected that hydrogen evolved due

to anoxic corrosion of metallic iron would diffuse into reactors containing nitrate contaminated aqueous solutions and act as the energy source for hydrogenotrophic anaerobic denitrifying microorganisms, thus effecting denitrification. As in case of the previous set of experiments, evolution of nitrate, nitrite and ammonia concentrations were monitored with time in all reactors to determine the extent of denitrification. However, no ammonia formation and very little nitrite production (less that 1 percent of total nitrogen added was converted to nitrite) were noticed in these reactors. Similar monitoring in a reactor containing 40 mg/L nitrate (as N) but no added microorganisms, but supplied with hydrogen as in the other reactors showed no degradation of nitrate, or formation of nitrite and ammonia. Evolution of nitrate in seeded reactors with the passage of time is presented in Figure 5.2. As per data presented in this figure, nitrate was completely removed within 50 days in reactors having initial nitrate concentration of 40, 80 and 120 mg/L. Approximately 90 percent of the nitrate was removed within 60 days in reactors having initial nitrate concentration of 160, 200 and 300 mg/L. The nitrate removal or denitrification rates were observed to be slower during these sets of experiments, as compared to previous experiments using external hydrogen supply (see Figure 5.1). This may be attributed to the fact that the microbial growth and hence denitrification was limited by the limited supply of hydrogen available in this case, as compared to the previous case, where hydrogen was not rate limiting. In that case microbial growth and hence rate of denitrification was only limited by the amount of electron acceptor, i.e., nitrate, nitrite etc., present in solution. Another difference between results presented in Figures 5.1 and 5.2 is the fact that no nitrite production was noticed in the latter case. This difference may be explained as follows. Rate of both nitrate and nitrite reduction was limited by the availability of these compounds in the first case (Figure 5.1), hydrogen being plentiful. Since the rate of nitrate reduction was faster than the rate of nitrite reduction under these conditions, this resulted in the accumulation of nitrite. In the second case (Figure 5.2), the rate of nitrate reduction was hydrogen limited, and hence considerably slower than in the previous case, while rate of nitrite reduction was not affected as severely. Under the circumstances, nitrite produced due to nitrate reduction was readily reduced further to $NO(g), N_2O(g),$ and $N_2(g)$, thus resulting in very little nitrite accumulation in the reactor.

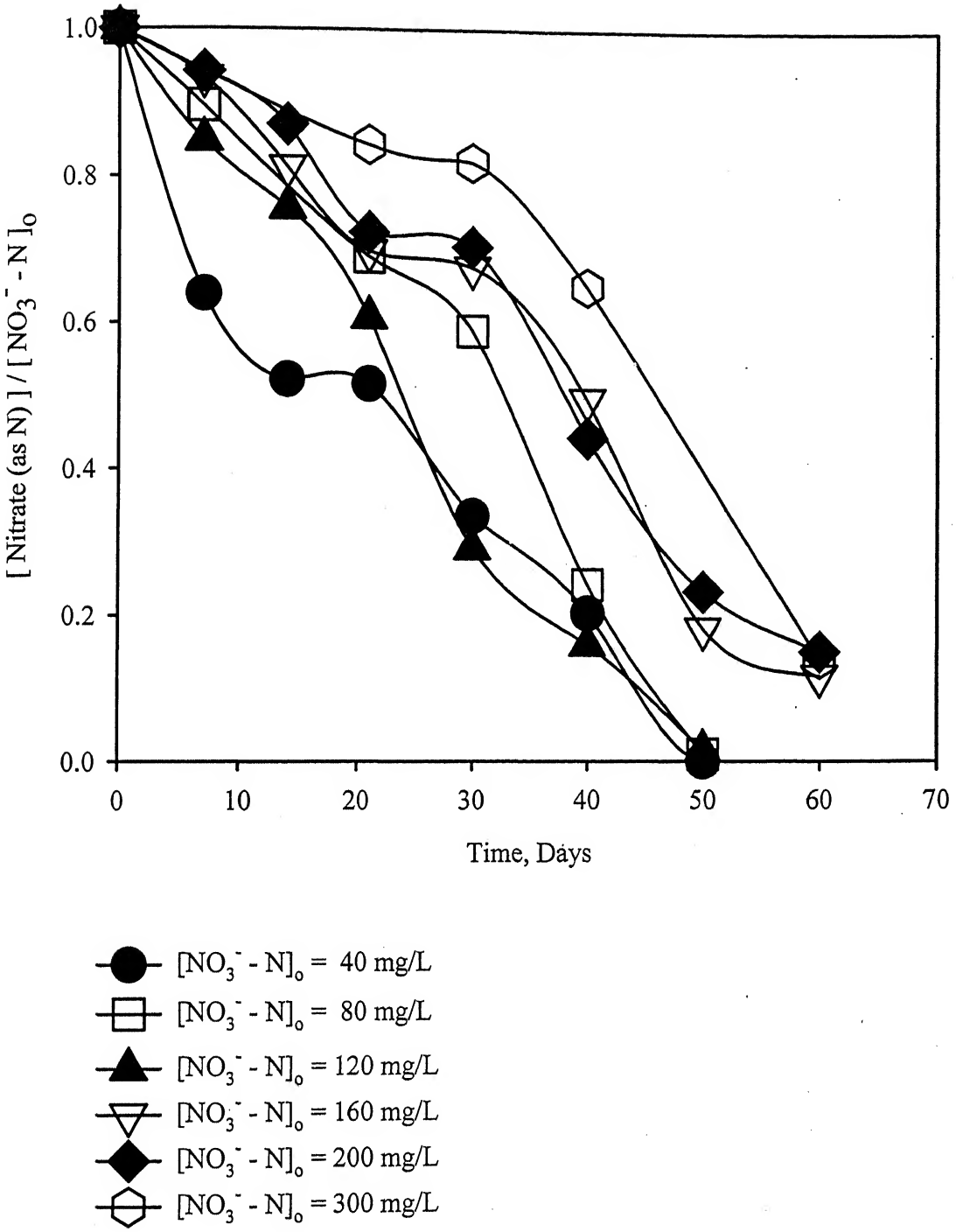

Figure 5.2 Reduction of Nitrate by Autotrophic Denitrification with Hydrogen Supplied by Anaerobic Corrosion of Metallic Iron
$[NO_3^- - N]_o$: Initial Nitrate Added

5.4 Influence of the Type of Metallic Iron

Based on the results presented in sections 5.2 and 5.3, it may be concluded that the hydrogenotrophic anaerobic denitrifying microorganisms isolated, cultured and maintained for this research were capable of denitrifying aqueous solutions contaminated with nitrate both using hydrogen supplied externally and hydrogen generated through anaerobic corrosion of metallic iron. The next objective of this research was to demonstrate the same denitrification process in a mixed system containing nitrate contaminated aqueous solution, metallic iron and hydrogenotrophic anaerobic denitrifying microorganisms. The choice of metallic iron type to be used for this purpose is of great importance. This is due to the abiotic reaction between metallic iron and nitrate, resulting in the formation of ammonia, an undesirable by-product of nitrate reduction (see Eq. 2.11). In other words, the chosen metallic iron type should be such that its direct abiotic reaction with nitrate is slow, thus producing little or no ammonia, while the iron undergoes anaerobic corrosion to produce the hydrogen required for sustenance of hydrogenotrophic anaerobic denitrifying microorganisms which are responsible for biological denitrification.

In order to determine the iron type most suitable for attainment of the above objective, abiotic nitrate reduction experiments using metallic iron were conducted, as described in section 4.3.4. Four types of metallic iron samples were tested for assessment of their suitability in this regard. Based on the results presented in Figure 5.3 it may be concluded that over the experimental duration of 6 days, 'steel-wool' was least able to reduce nitrate, while all other iron types showed considerably greater ability to reduce nitrate to nitrite and ultimately ammonia. Based on the results of this experiment, 'steel-wool' was chosen as the iron type to be used for all successive experiments in this research. The reason for 'steel-wool' being the least effective in reducing nitrate to ammonia appears to be the low surface area to weight ratio of this substance as compared to other iron types like 'iron powder' or 'iron filings'. As per values reported by Till et al. (1998), typical acid-washed metallic 'iron powder' was reported to have surface area to weight ratio of 2 m^2/g, as compared to 0.0075 m^2/g for 'steel wool'. Since the rate of reaction between metallic iron and nitrate is a function of metallic iron surface area, thus

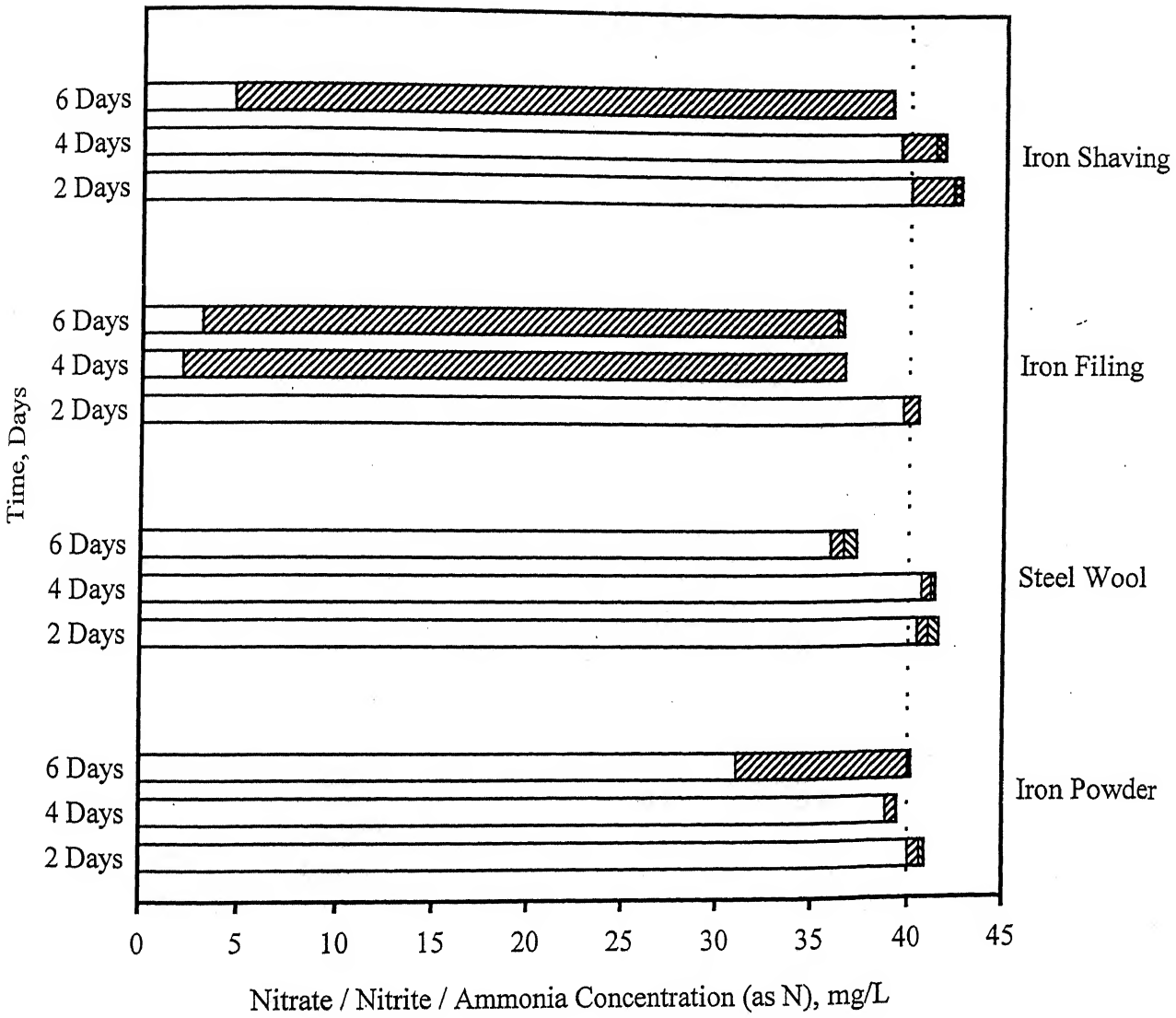

Figure 5.3 Extent of Abiotic Nitrate Reduction by Metallic Iron of Various Types
(Iron Quantity: 1 g; Reactor Volume: 300 mL)

for the same quantity, steel wool will interact far less with nitrate and hence produce less ammonia. An undesirable corollary of the above observation is the fact that 'steel wool' is also likely to corrode more slowly through interaction with water due to the lower surface area it offers in comparison to other iron types. This would imply a decline in hydrogen production by anaerobic corrosion, resulting in the retardation of biological denitrification reactions due to the prevalence of hydrogen limiting conditions in the system.

5.4 Influence of Metallic Iron Concentration

Based on experimental results described in Figure 5.4, it may be concluded that among the various iron types tested, 'steel wool' is the least effective in directly reducing nitrate to ammonia. Hence, it is most suitable for use as a facilitator of biological denitrification using hydrogenotrophic anaerobic denitrifying microorganisms. However, questions still remain regarding the amount of steel wool to be used such that ammonia formation is minimized, while a reasonable rate of biological denitrification is sustained. It is to answer these questions that the next set of experiments were conducted.

These experiments involved adding 2, 3, 4 and 5 g of 'steel wool' in 300 mL volume reactors containing aqueous solution contaminated with 40 mg/L nitrate (as N). Two reactors were prepared for each iron amount, out of which one was seeded with hydrogenotrophic anaerobic denitrifying microorganisms, while the other was maintained in sterile condition. Experiments were conducted according to the protocol specified in section 4.3.4. Evolution of nitrogen species, i.e., nitrate, nitrite and ammonia in these reactors were monitored after 7 and 14 days. Results obtained after 7 days are shown in Figure 5.4. Similar results were also obtained after 14 days. Based on the results presented in Figure 5.4, the following may be concluded. First, the total aqueous nitrogen concentration, i.e., the sum of nitrate, nitrite and ammonia, expressed as nitrogen, remained unchanged in the sterile (abiotic) reactors at approximately 40 mg/L (as N). Second, the total nitrogen concentration as defined above, declined in the seeded (biological) reactors. This may be attributed to biological denitrification in these reactors. Third, ammonia formation was noticed in both sterile and seeded reactors, with

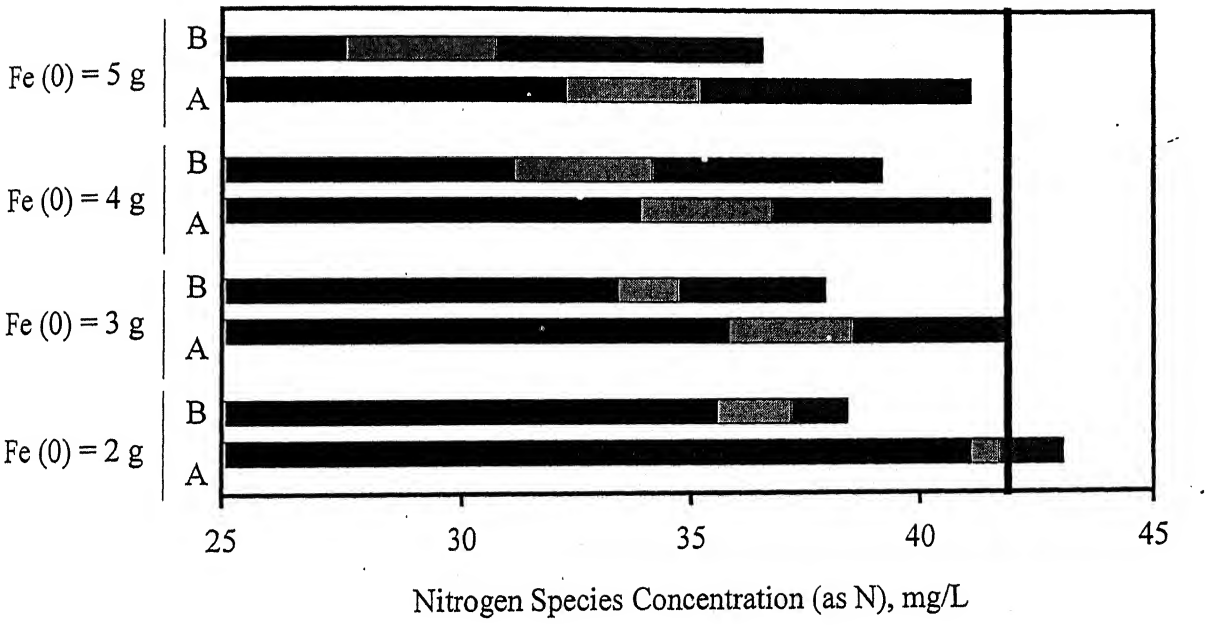

Figure 5.4 Evolution of Nitrate, Nitrite and Ammonia due to Abiotic and Biological Nitrate Reduction in Batch Reactors for 7 Days (Reactor Volume: 300 mL)

the amount of ammonia formed increasing with increasing 'steel-wool' concentration in the reactors. Fourth, in the seeded reactors, the extent of biological denitrification increased very slightly with increase in iron concentration in the reactor. Fifth, even at the lowest metallic iron concentration tested, i.e., 2 g, considerable amount of ammonia formation was noticed in both seeded and sterile reactors. Based on these results, it seems probable that employing a lower metallic iron concentration in subsequent biological denitrification experiments of similar type may be necessary to achieve lesser ammonia formation and hence better end-product distribution.

5.5 Metallic Iron Assisted Autotrophic Denitrification in Batch Reactors

Results described in the previous section indicated that even with 'steel-wool' concentration of 2 g in a 300 mL reactor, considerable amount of ammonia formation is possible during biological denitrification reactions. This is due to the competitive kinetics between the abiotic and biological mode of nitrate reduction, where abiotic reduction results in the formation of ammonia. One way to reduce ammonia production is to further decrease the 'steel-wool' concentration in the reactors, thus reducing abiotic interaction between 'steel-wool' and nitrate which results in ammonia formation. As mentioned earlier, such a course of action may also reduce hydrogen formation, thus affecting biological denitrification adversely. However results presented in Figure 5.4 indicated that reduction in iron quantity may not affect denitrification rates in a precipitous manner.

Based on the above reasoning, efforts were made to determine long term biological denitrification characteristics in batch reactors containing 0.5, 1.0 and 1.5 g 'steel-wool'. Experiments were conducted according to the protocol specified in section 4.3.4. Evolution of nitrogenous species, i.e., nitrate, nitrite and ammonia in these reactors were monitored at 7, 14, 21, 28, 30, 40, 50 and 60-day intervals. Results obtained for the three cases tested, i.e., using 0.5, 1.0 and 1.5 g iron are shown in Figure 5.5. These results indicate that for all three cases nitrate concentration in the reactor was nearly reduced to zero after 60 days. Formation and subsequent disappearance of nitrite was also noticed in all cases. Ammonia formation was low (< 5 mg/L as N) up to 40 days in all reactors,

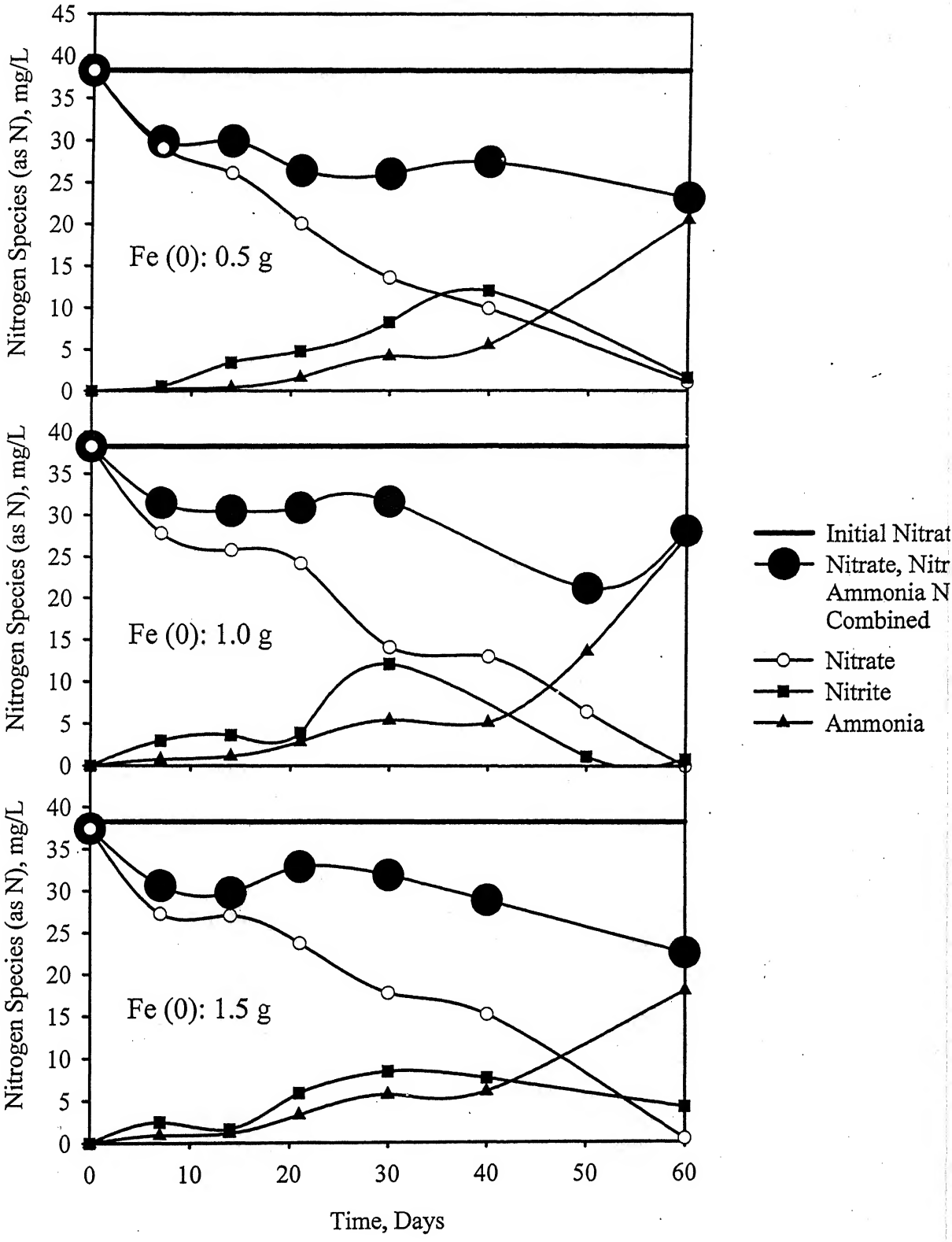

Figure 5.5 Decrease in Total Nitrogen Concentration in a Batch Reactor Containing Steel Wool, Nitrate and Autotrophic Denitrifying Microorganisms
(Reactor Volume: 300 mL)

after which a sharp increase in ammonia concentration was observed. Extent of denitrification in all reactors was approximately the same, with the total aqueous nitrogen concentration declining from approximately 40 mg/L to approximately 25 mg/L in 60 days. Following conclusions may be drawn from the results presented in Figure 5.5. First, despite relatively low metallic iron concentration in these reactors, nitrate reduction was not hampered, as seen from the reduction of nitrate concentration to below detection levels. Second, nitrate reduction was only partially biological, as seen from the buildup of ammonia in all reactors. It appears that for the initial reaction period of up to 40 days, the nitrate reduction was mostly biological, as seen by the low ammonia concentration in reactors up to that time. Beyond 40 days, decreased nitrate concentration in the reactors reduced the rate of biological denitrification process and hence abiotic nitrate reduction process gained precedence. This is demonstrated by steep increase in ammonia production in all reactors after 40 days.

5.6 Metallic Iron Assisted Autotrophic Denitrification in Up-Flow Column Reactors

The final objective of this research involving autotrophic denitrification was to develop a reactive porous medium containing metallic iron and denitrifying microorganisms for removal of nitrate in a flow through system. As mentioned before, such a medium can conceivably be used in 'in-situ' sub-surface barriers placed across groundwater flows for remediation of nitrate contaminated groundwater resources. Based on batch studies described in the previous section, reactive media of varying compositions were prepared by mixing 125 cm^3 of sterilized, acid washed sand with 0.5, 1.0 and 1.5 g of 'steel-wool', which was the metallic iron source. Media prepared thus was loaded into columns as described in chapter IV, section 4.3.5, and seeded with denitrifying microorganisms, if necessary. The experimental protocol adopted for carrying out various experiments using these columns is described in detail in section 4.3.5.

Two experiments were conducted with unseeded columns maintained in sterile conditions by addition of $HgCl_2$. One of these columns contained only sand and the other sand mixed with 1.0 g of 'steel-wool'. The influent nitrate concentrations in both columns

were approximately 33 mg/L (as N). These columns were operated in up-flow mode, and a predetermined volume of water was withdrawn from the columns each day, thus controlling the retention time of water in the columns. Initial rate of effluent extraction from the columns corresponded to a retention time of 1.3 days. The rate of effluent extraction was progressively decreased with time such that the retention time of water in the columns increased in steps to a maximum value of 13 days. The reactors were maintained at this retention time for a period of 12 days starting from the twenty-fourth day after the start of effluent extraction. Effluent nitrate and ammonia concentrations from both columns were measured every few days. However, effluent nitrite concentrations could not be measured due the increased chloride concentration in the effluent, resulting from addition of $HgCl_2$. This resulted in the nitrite peak in the ion chromatograph being swamped by the chloride peak. The results of the two experiments described above are shown in Figure 5.6. In case of the column containing only sand, ammonia concentration in the effluent was found to be very low for all retention times (1.3 – 13 days) tested. This is as per expectation, since no obvious nitrate reduction and hence ammonia formation mechanism existed in this column. In case of the column containing both sand and metallic iron, ammonia concentration in the effluent was much higher than in the previous column for all retention times. This is consistent with the possibility of nitrate reduction to ammonia in presence of metallic iron. It was also observed that ammonia concentration in the effluent from this column showed a general upward trend with increase in retention time of water inside the column. This is also easily explicable, considering that more retention time affords more opportunity for abiotic nitrate reduction, as described before.

Three experiments were conducted using columns seeded with autotrophic denitrifying microorganisms. The 'steel-wool' concentration in these columns was 0.5, 1.0 and 1.5 g respectively. The influent nitrate concentrations, mode of operation and sample collection techniques in these columns were similar to what was described in case of sterile columns. In addition to effluent nitrate and ammonia concentrations, nitrite concentration was also measured in the effluent from these columns, though the nitrite concentration was found to be negligibly small in most cases. Total nitrogen balance for

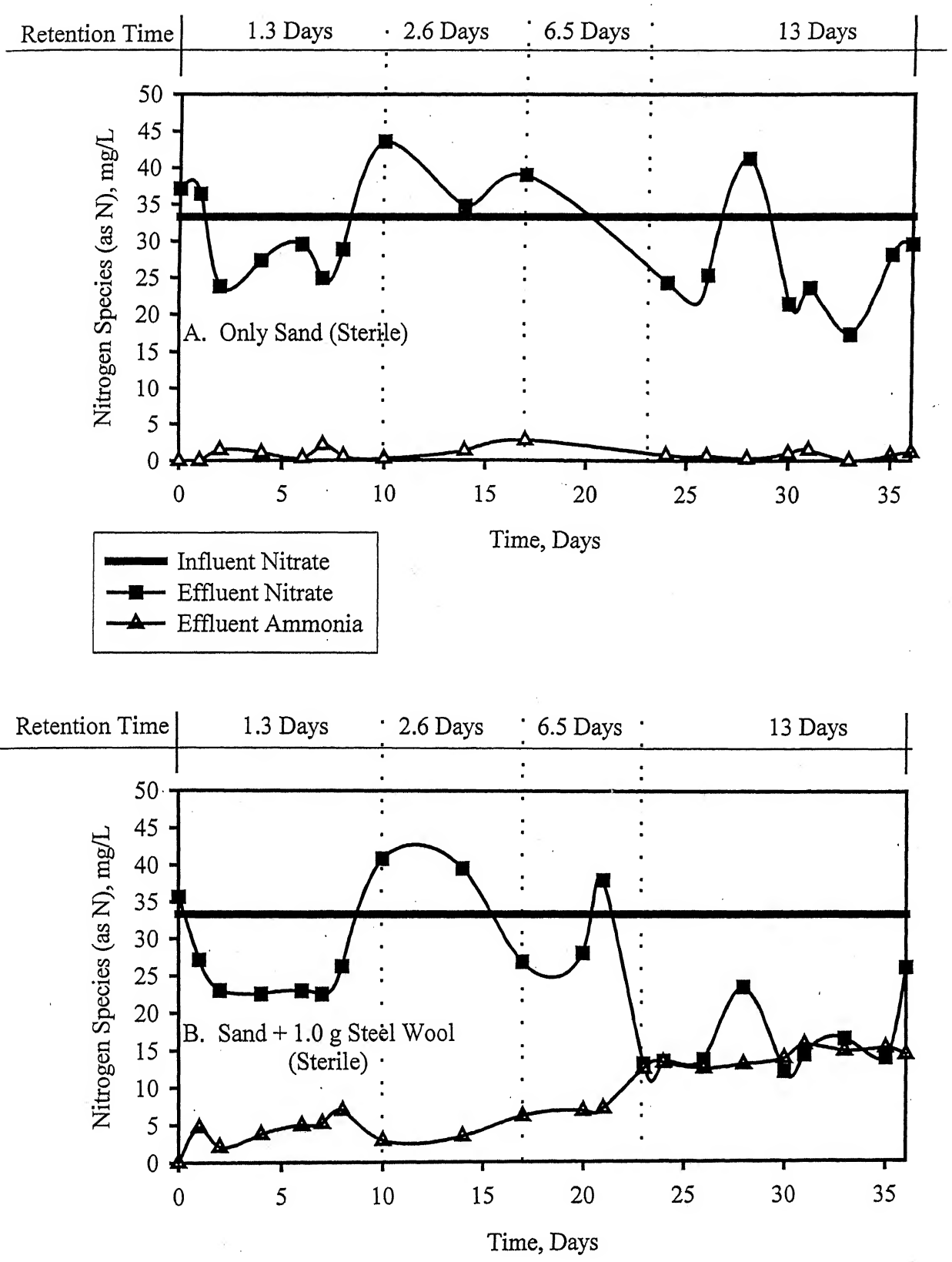

Figure 5.6 Abiotic Nitrate Reduction In the Presence of Metallic Iron in Intermittent-Flow Upflow Column Reactor
(Volume of Sand: 125 cm^3)

the effluent from the column was also performed in all cases, such that this value and the influent nitrate concentration could be compared for determination of the extent of biological denitrification. The results of the three experiments described above are shown in Figure 5.7. Based on these results, it appears that provision of a retention time of 13 days in the reactive media was sufficient for reduction of nitrate concentration in the effluent to approximately zero in all three cases studied. However, in all three cases, the effluent contained substantial amounts of ammonia, which is an undesirable by-product of nitrate reduction. Perusal of the trend in effluent ammonia concentration with increase in retention time for all three cases presented in Figure 5.7 indicate that increasing retention time beyond 13 days is unlikely to provide a better end-product distribution. Also, it was observed in the same figures that fraction of initial nitrate being converted to ammonia by abiotic nitrate reduction increases with increasing iron concentration in the reactive media.

5.7 General Discussion of Results

Based on the results presented in Figure 5.7 and subsequent discussion of the results, important conclusions may be drawn regarding the optimal composition of reactive media required for autotrophic biological denitrification. It appears that to produce effluent with lower ammonia concentration than those exhibited in Figure 5.7, the 'steel-wool' concentration of the reactive media has to be lowered even below the lowest value (0.5 g) tested during this research. Reduction of 'steel-wool' concentration as suggested above would, however, also reduce the rate of hydrogen production, which is undesirable from the biological denitrification perspective. It is entirely possible that lower metallic iron concentration in the reactive media may result in increased nitrate concentration in the effluent in lieu of the decline in ammonia concentration. Preliminary indications of this eventuality can be obtained through careful observation of the effluent nitrate data for the three cases presented in Figure 5.7. In the case where metallic iron concentration is the lowest (0.5 g), the steady-state effluent nitrate concentration corresponding to retention time of 13 days is seen to be higher than that in cases where metallic iron concentration was 1.0 and 1.5 g. The higher nitrate concentration in the effluent as hypothesized above can of course be partially or fully reduced by increasing the retention time. However, as discussed earlier, increasing the retention time also increases the

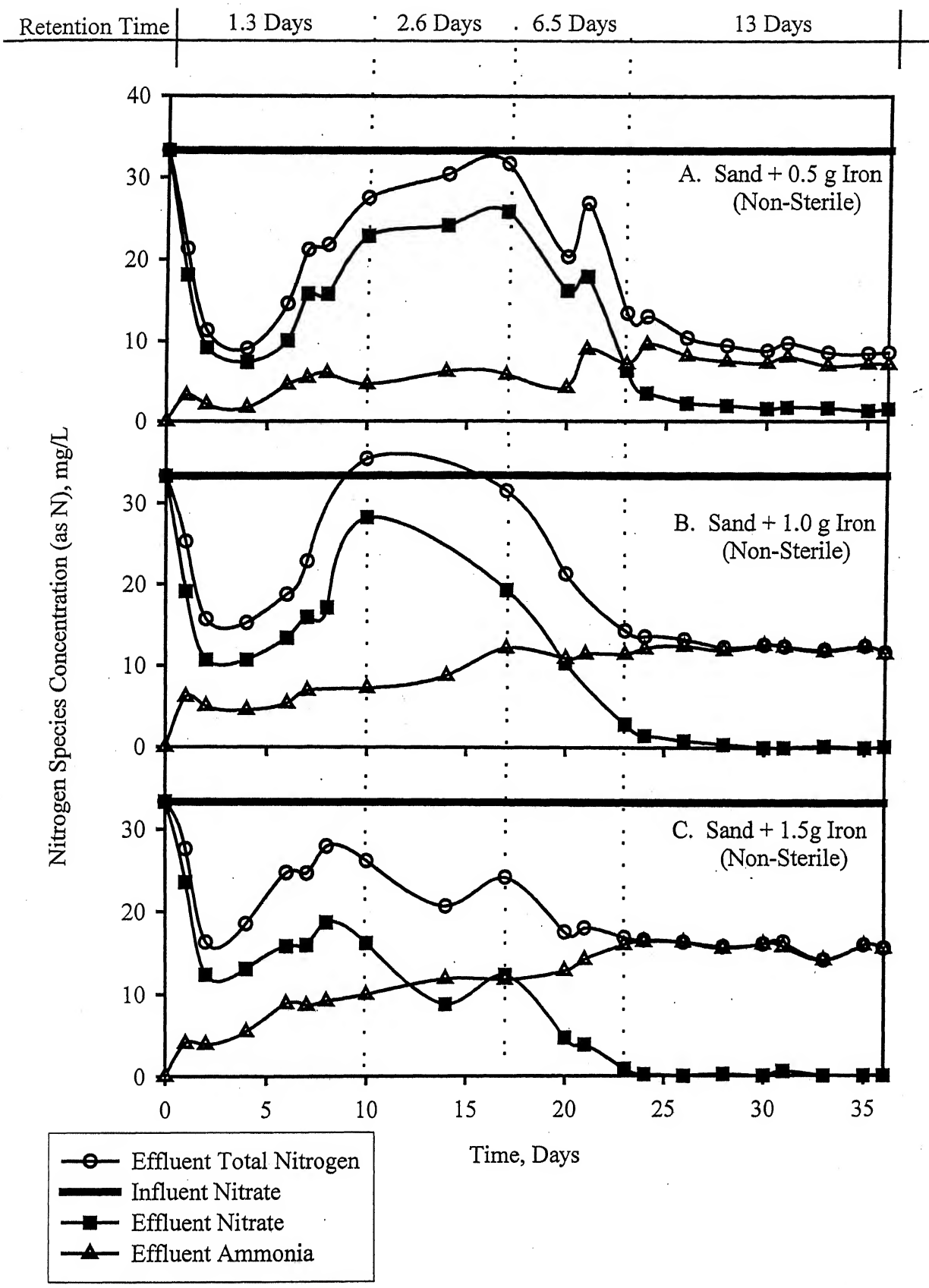

Figure 5.7 Biological Nitrate Reduction in the Presence of Metallic Iron in Intermittent-Flow Upflow Column Reactor
(Volume of Sand: 125 cm^3)

possibility of producing more ammonia in lieu of reducing the nitrate concentration. Determination of optimal composition of the reactive media for denitrification thus remains an unfinished task, though clear pointers regarding probable composition of such media and retention time required to achieve denitrification in such media have been obtained through the present study.

In more general terms, it appears that metallic iron assisted autotrophic denitrification is possible in flow-through systems containing porous media. However, results also suggest that retention time of 13 or more days may be required for complete denitrification of nitrate-contaminated water using the process under consideration. Provision of such a large retention time is impractical in unit processes used for pollutant removal in water or wastewater treatment. However, such a process may be ideal for application in sub-surface 'in-situ' remediation systems for removal of nitrate from groundwater, considering that groundwater flow velocity is very low. One such system under consideration at the present time is the so-called 'in-situ reactive barrier' system, where a semi-pervious barrier containing the reactive material, i.e., sand and metallic iron, seeded with denitrifying microorganisms, is placed across the groundwater flow-path in the subsurface through trenching/filling. As groundwater flows through the reactive barrier, denitrification takes place and the water on the downstream side of the barrier has lower nitrate concentration. Considering groundwater flow rate to be in the order of 10-40 cm/day, approximate thickness of a barrier of this type is likely to be 260 – 1040 cm (assuming porosity of barrier material to be 0.5) for a retention time of 13 days.

CHAPTER VI
SUMMARY AND CONCLUSIONS

To summarize, based on the results of this research, it appears that under favorable conditions, metallic iron assisted autotrophic denitrification is possible during flow through porous media. This concept may find application in the design and construction of 'in-situ reactive barrier' systems for removal of nitrate from groundwater. However, before more definitive conclusions in this regard can be made, more research in the area is necessary. Inquiry may be channeled in various directions, and may include extension of work presented in this dissertation concerning determination of optimum composition of reactive media for obtaining more favorable end-product distribution. Another important area of research is the problem of long-term pH increase in reactive media. This occurs due to the release of OH^- ions during the biological denitrification process, which may raise the pH of the reactive media to values beyond the limit for sustenance of denitrifying microorganisms. To counter this effect, inherent pH buffering capacity of the reactive media must be increased through the amendment of the reactive media with suitable additives. Another possible cause of concern is the release of ferrous ions as a consequence of the biological denitrification process. However, measurement of iron concentration in the effluent from the reactive media during this research (data not shown) indicated that dissolved iron concentration is rarely more that 2 mg/L, even when nitrogen removal by biological denitrification is 30 mg/l (as N) or higher. This suggests that dissolved iron released due to corrosion of metallic iron is precipitating as amorphous $Fe(OH)_2$ inside the reactive media. Such precipitation and progressive growth of microbial biomass inside the reactive media may result in reduction of porosity of the reactive media, thus affecting the long-term efficiency of such media in regard to denitrification capacity. However, more information on this subject is required before anything more definitive can be said.

Despite various limitations and need for further research as outlined above, the research presented in this dissertation represents a significant advancement in the current 'state-of-the-art' on the subject of metallic iron assisted biological denitrification. As mentioned

before, the main motivation behind this research was to determine the optimum reactive media composition for effecting nitrate reduction with favorable end-product distribution, i.e., to convert nitrate to nitrogen gas through biological denitrification, while restricting formation of ammonia through abiotic nitrate reduction. In more specific terms, the main findings of the research described in this dissertation are the following.

- It was demonstrated that hydrogenotrophic anaerobic denitrifying microorganisms used in this study have the ability to denitrify solutions contaminated with nitrate using hydrogen supplied from an external source, or using hydrogen generated through anaerobic decomposition of metallic iron. Under conditions of abundant hydrogen supply, nitrate removal resulted in the formation and accumulation of nitrite as an intermediate-product. However the accumulated nitrate was further degraded and removed from solution with the passage of time.
- The choice of metallic iron type to be used for hydrogen supply in a mixed system containing nitrate, metallic iron and hydrogenotrophic anaerobic denitrifying microorganisms was of great importance due to the abiotic reaction between metallic iron and nitrate resulting in the formation of ammonia. Four types of metallic iron samples, 'iron powder', 'iron shaving', iron filing' and 'steel wool' were tested for extent of ammonia formation, and 'steel-wool' was found to produce least ammonia and was thus used for subsequent studies.
- To answer questions regarding the amount of 'steel-wool' to be used such that ammonia formation is minimized, while a reasonable rate of biological denitrification is sustained, preliminary experiments were conducted with different concentrations of 'steel-wool' in 300 mL volume batch reactors. Results of these experiments indicated that further batch studies should be conducted with 0.5, 1.0 and 1.5 g of 'steel-wool', in similar 300 mL reactors to further elucidate the metallic iron assisted denitrification process.
- In subsequent experiments conducted with metallic iron concentrations as above, nitrate concentration was reduced from 40 mg/L (as N) to nearly zero after 60 days in all three cases. In addition, formation and subsequent disappearance of nitrite was observed, and ammonia formation was low (< 5 mg/L as N) up to 40 days, after

which a sharp increase in ammonia concentration was observed. Extent of denitrification in all reactors was approximately forty percent after 60 days.

- The final objective of this research was to develop a reactive porous media containing metallic iron and denitrifying microorganisms for removal of nitrate in a flow through system. For this purpose, three experiments were conducted using appropriately seeded columns with 'steel-wool' concentrations of 0.5, 1.0 and 1.5 g respectively. Based on results, it appears that provision of a retention time of 13 days in the reactive media was sufficient for reduction of nitrate concentration in the effluent to approximately zero in all three cases studied. However, in all three cases, the effluent contained substantial amounts of ammonia, which is an undesirable by-product of nitrate reduction.

In conclusion, based on the results of this research, it appears that to achieve even better end-product distribution than obtained in the column studies described here, the 'steel-wool' concentration in the reactive media has to be lowered even below lowest value (0.5 g) tested during this research. This will ensure reduction in ammonia concentration in the effluent. To counter any detrimental effect of lowered metallic iron concentration on nitrate removal, increase of the retention time of water in the reactive media to values higher than 13 days may be required.

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